AD_____

Award Number: DAMD17-99-1-9546

TITLE: Kainate Receptors in the Striatum: Implications for Excitotoxicity in Huntington's Disease

PRINCIPAL INVESTIGATOR: Yoland Smith, Ph.D.

CONTRACTING ORGANIZATION: Emory University
Atlanta, Georgia 30322

REPORT DATE: August 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2000	Annual (15 Jul 99 – 14 Jul 00)

4. TITLE AND SUBTITLE
Kainate Receptors in the Striatum: Implications for Excitotoxicity in Huntington's Disease

5. FUNDING NUMBERS
DAMD17-99-1-9546

6. AUTHOR(S)
Yoland Smith, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Emory University
Atlanta, Georgia 30322

E-MAIL:
yolands@rmy.emory.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; Distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

Huntington's disease (HD) is a neurodegenerative condition characterized by a loss of projection neurons in the striatum. Although various hypotheses have been proposed to explain the mechanisms that underlie the striatal neuronal death, excitotoxicity still deserves major interest. Recent findings indicate that changes in the genotype of the kainate receptor subunit, GluR6, are associated with variation in the age of onset of HD, which implicates the kainate receptors in the pathogenesis of HD. The rationale of this project is that pre-synaptic kainate receptors control the release of glutamate from cortical or thalamic terminals, and that an abnormal regulation of these receptors is involved in the death of striatal neurons in HD. We, therefore, propose to use state-of-the-art electron microscope techniques to test a series of hypotheses that will help to elucidate the localization and understand better the role of kainate receptors in the primate striatum. The results of these studies will provide a strong basis for studying the potential mechanisms by which these receptors participate in the death of striatofugal neurons in HD. Moreover, they will help the development of novel therapeutic strategies aimed at targeting pre-synaptic kainate receptors in HD and other basal ganglia disorders.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Neurotoxin	102
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION:

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by the death of striatal neurons. Chorea is the most common involuntary movement in patients who suffer of HD. This could be combined with cognitive and memory deficits at a later stage of the disease. The HD mutation was identified in 1993 as an unstable expansion of CAG (trinucleotide) repeats on the gene which encodes the protein "Huntingtin" on chromosome 4. In more than 60% of HD patients, there is a high degree of inverse correlation between the number of CAG repeats and the age of onset of the disease or degree of striatal degeneration (Vonsatell and DiFiglia, 1998). However, about 15% of the HD cases of which the age of onset cannot be explained by the CAG repeats, were found to have mutations in the gene encoding the GluR6 subunit of the glutamatergic kainate receptor (Rubinztein et al., 1997; MacDonald et al., 1999) which highlight the importance of those receptors in the pathogenesis of the striatum in HD. Although the existence of kainate receptors has long been established, little is known about their functions and distribution in the central nervous system. Previous data obtained in our laboratory showed that the kainate receptor subunits GluR6/7 are strikingly enriched in the monkey striatum but, in contrast to other ionotropic glutamate receptors which are found almost exclusively at postsynaptic sites, the GluR6/7 kainate receptor subunits are strongly expressed pre-synaptically in glutamatergic terminals. The objective of this project is to further this analysis and provide a detailed map of the synaptic localization of kainate receptor subunits in the striatum of monkeys so that we understand better the mechanisms by which these glutamate receptors might mediate the death of striatal neurons in HD.

BODY:

The past year has been very fruitful for this project. So far, Specific Aims #1,2,3 and 5 have been completed and results will be shortly submitted for publication in The Journal of Neuroscience. These findings will be presented at the next Society for Neuroscience Meeting in New Orleans (appendix 1) and are part of a recent review we submitted for publication to The Journal of Chemical Neuroanatomy (appendix 2). The results obtained are illustrated in Figs. 1-10.

As mentioned in the original application, the pattern of striatal neuronal degeneration is heterogeneous in HD. For instance, the tail of the caudate nucleus is usually more affected than the body which, in turn, is more involved than the head. Similarly, the caudal portion of the putamen is more affected than the rostral putamen. One of the specific aim of our application was to test the possibility that areas which degenerate first contain a larger density of kainate

receptor-immunoreactive terminals. To do so, we processed striatal sections for GluR6/7 and KA2 immunoperoxidase labelling and sampled various striatal regions for the presence of immunoreactive elements. As expected based on our previous study (Charara et al., 1999), the immunoreactivity for both kainate receptor antibodies was found in pre- and postsynaptic elements, including putative glutamatergic axon terminals. However, quantitative measurements of the relative abundance of labeled structures did not reveal any significant difference in the density of immunoreactive terminals between striatal regions with a low and high degeneration rate (Fig. 1).

The second part of this study was to elucidate the subsynaptic localization of GluR6/7 and KA2 immunoreactivity. To reach this objective we used both pre- and post-embedding immunogold methods. In material stained with the pre-embedding immunogold technique, gold particles were associated with the endoplasmic reticulum in perikarya of both projection neurons and interneurons (Fig. 2). Immunostaining was also found in dendrites, spines and axon terminals forming asymmetric synapses (Fig. 3). A common feature that characterized the pre- and postsynaptic labelling was that gold particles were largely intracellular rather than being attached to the plasma membrane (Figs. 3, 4A,C). We, then, determined the subsynaptic localization of the membrane-bound gold particles for GluR6/7 and KA2. In this analysis, the labelling was categorized as extrasynaptic if it was attached to parts of the plasma membrane not involved in synaptic contacts, perisynaptic if it was located at the edges (less than 20 nm from) of symmetric or asymmetric postsynaptic specializations and synaptic if it occurred in the main body of symmetric or asymmetric synapses. There was no significant difference in the pattern of distribution of GluR6/7 and KA2 immunoreactivity. Overall, almost 70% of membrane-bound immunoreactivity for both receptor subunits was located extrasynaptically, whereas 10-15% was expressed synaptically either in the main body of asymmetric post-synaptic specializations (in spines and dendrites) or in the pre-synaptic grid of asymmetric axo-spinous synapses (in terminals) (Figs 3, 4B,D). This large pool of extrasynaptic receptors raises questions about the functions and mechanisms of activation of these receptors. Spillover of neurotransmitter from the synaptic cleft of glutamatergic synapses and glutamate release from glia are two potential sources of activation of these receptors (Asztely et al., 1997; Antanitus, 1998).

To further characterize the subsynaptic localization of kainate receptor subunits, we processed tissue for the post-embedding immunogold localization of GluR6/7 and KA2. Data obtained in these experiments are illustrated in figures 5-8. In brief, the main findings are: (1) As was found in the immunoperoxidase material (Charara et al., 1999), axon terminals forming asymmetric synapses display a stronger immunoreactivity for both receptor subunits than spines (Fig. 6), (2) The pre-synaptic labelling was often attached to the membrane of vesicular structures inside axon terminals forming asymmetric axo-spinous synapses (Fig. 5C,F). No

particular relationships were found between the labeled vesicles and their distance from the presynaptic grid (Fig. 8), i.e. in some instances the labelling was confined to the presynaptic grid (Fig. 5D-E) while in others it could be located as far as 1.0 μm away from the synapse (Fig. 5C,F), (3) In dendrites and spines, the immunogold particles were largely expressed extrasynaptically though synaptic and perisynaptic labelling at asymmetric synapses was frequently encountered (Fig. 7). We also commonly found aggregates of gold particles associated with the postsynaptic densities of asymmetric synapses (Fig. 5D).

Finally, the last objective of this study was to elucidate the sources of kainate receptor containing terminals in the striatum. To do so, we combined the anterograde transport of biotinylated dextran amine (BDA) and GluR6/7 or KA2 immunoreactivity. BDA was injected either in the centromedian nucleus (CM) or the primary motor cortex (MI) to label extensively the two major sets of glutamatergic terminals which form asymmetric synapses in the postcommissural putamen. Sections were processed for the double electron microscopic labelling of BDA (peroxidase) and GluR6/7 or KA2 (pre-embedding immunogold). Following both injections, 50-70% of anterogradely labelled terminals displayed kainate receptor subunit immunoreactivity (Figs. 9-10), which indicates that kainate receptors may act as autoreceptors to modulate the release of glutamate from thalamic and cortical afferents to the sensorimotor striatum in monkeys.

KEY RESEARCH ACCOMPLISHMENTS:

So far, the main findings of this project are:

(1) The relative abundance of glutamatergic terminals immunoreactive for kainate receptor subunits does not vary throughout the striatum despite the fact that some striatal regions are more sensitive than others to degeneration in Huntington's disease.
(2) The bulk of pre- and postsynaptic GluR6/7 and KA2 immunoreactivity is expressed intracellularly under basal conditions.
(3) Most of the membrane-bound labelling for GLUR6/7 and KA2 is expressed extrasynaptically though synaptic and perisynaptic labelling of glutamatergic synapses is also encountered.
(4) In immunoreactive terminals, GluR6/7 and KA2 labelling is associated with the membrane of vesicles which are randomly distributed relative to the pre-synaptic grid of asymmetric synapses.
(5) More than half of cortical and thalamic inputs from the primary motor cortex and the centromedian nucleus, respectively, express GluR6/7 and KA2 immunoreactivity in the postcommissural putamen.

REPORTABLE OUTCOMES:

Kieval, J.Z., A. Charara, J.-F. Paré and **Y. Smith** (2000) Subcellular localization of kainate receptors in the monkey striatum. Soc. For Neursoci. Abstr. ID 2528.

Smith, Y., A. Charara, M. Paquet, J.Z. Kieval, J.E. Hanson, W.G. Hubert, M. Kuwajima and A.I. Levey (2000) Ionotropic and Metabotropic GABA and Glutamate Receptors in the Primate Basal Ganglia. J. Chem. Neuroanat. (submitted for publication).

Kieval, J.Z., A. Charara, J.-F. Paré and **Y. Smith** (???) Subcellular and subsynaptic localization of kaiante receptor subunit immunoreactivity in the monkey striatum. (in preparation).

CONCLUSIONS:

Three main conclusions can be drawn from data obtained so far in this project: (1) *There is no differential distribution of kainate receptor subunit-containing terminals between striatal regions sensitive and resistant to neurodegeneration in Huntington's disease*. These data suggest that the potential implication of kainate receptors in the excitotoxic phenomenon that underlies striatal cell death in HD is unlikely to be the result of a larger density of malfunctioning presynaptic kainate receptors. However, this does not rule out the importance of kainate receptors in HD pathology. Although the kainate receptor density is the same throughout the striatum, their pharmacological properties might be altered and lead to an inefficient regulation of glutamate release in the striatum of Huntington's patients, (2) *The bulk of kainate receptor subunit immunoreactivity is located either intracellular or at extrasynaptic sites along neuronal plasma membrane*. This suggests that, under basal conditions, kainate receptors are unlikely to mediate fast synaptic communication in the primate striatum. This is in line with recent data showing that stimulation of glutamatergic afferents does not induce synaptic activation of kainate receptors in slices of rat striatum (Chergui et al., 2000). This is also consistent with recent evidence showing that the kainate receptor-mediated presynaptic control of GABA release in the hippocampus is a much slower process than activation of other ionotropic glutamate receptors and involves a metabotropic function (Rodriguez-Moreno and Lerma, 1998). In line with these data, it is noteworthy that the pattern of distribution of kainate receptor subunits described in this report resembles that of metabotropic glutamate receptors (Hanson and Smith, 1999; Smith et al., 2000a,b). Further studies are essential to better characterize the functional significance of these anatomical data, (3) *Terminals from both the centromedian thalamic nucleus and the primary*

motor cortex display kainate receptor subunit immunoreactivity. These observations indicate that kainate may act as autoreceptors to modulate the release of glutamate from both the corticostriatal and thalamostriatal pathways in the sensorimotor striatal territory. Whether kainate receptor activation induces a facilitation or inhibition of glutamate release from these terminals remains to be established. Functional data obtained so far indicate that pre-synaptic kainate receptors may generate facilitatory or inhibitory effects on transmitter release in other brain regions (Rodriguez-Moreno and Lerma, 1998; Chergui et al., 2000).

REFERENCES:

Antanitus, D.S. (1998) A theory of cortical neuron-astrocyte interaction. Neuroscientist 4: 154-159.

Asztely et al. (1997) Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake. Neuron 18, 281-293.

Charara, A., E. Blankstein and Y. Smith (1999) Presynaptic kainate receptors in the monkey striatum. Neuroscience 91: 1195-1200.

Chergui, K. et al. (2000) Functional GluR6 kainate receptors in the striatum: Indirect downregulation of synaptic transmission. J. Neurosci. 20: 2175-2182.

Hanson, J.E. and Y. Smith, Y. (1999) Group I metabotropic glutamate receptors at GABAergic synapses in monkeys. J. Neurosci. 19: 6488-6496.

Liu, Q.-S. et al. (1999) Kainate acts at presynaptic receptors to increase GABA release from hypothalamic neurons. J. Neurophysiol. 82: 1059-1062

McDonald, M.E. et al (1999) Evidence for the GluR6 gene associated with younger onset age of Huntington's disease. Neurology 53: 1330-1332.

Rodriguez-Moreno, A. and J. Lerma (1998) Kainate receptor modulation of GABA release involves a metabotropic function. Neuron 20: 1211-1218.

Rubinsztein, D.C. et al. (1997) Genotypes at the GluR6 kainate receptor locus are associated with variation in the age of onset of Huntington's disease. Proc. Natl. Acad. Sci. USA 94: 3872-3876.

Smith, Y., A. Charara, J.E. Hanson, M. Paquet and A.I. Levey (2000) GABA-B and group I metabotropic glutamate receptors in the striatopallidal complex in primates. J. Anatomy 196: 555-576.

Vonsattel, J.P.G. and Di Figlia, M. (1998) Huntington Disease. J. Neuropathol. Exp. Neurol. 57: 369-384 .

FIGURE LEGEND:

Fig. 1: Relative distribution (A,C) and photomicrographs (B,D) of GluR6/7- (A,B) and KA2- (C,D) immunoreactive elements in various striatal regions in monkeys. In B and D, Te indicates immunoreactive terminals whereas the asterisks label non-immunoreactive boutons. Ax: Axon; De: Dendrite. Scale bars: 0.5 μm

Fig. 2: GluR6/7-containing neuronal perikaryon in the monkey putamen. The framed area in B is shown at higher magnification in A. Note aggregates of gold particles associated with membrane of the endoplasmic reticulum in A and C. Scale bars: A: 0.25 μm; B: 1.0 μm; C: 0.5 μm.

Fig. 3: Subcellular localization of GluR6/7 (A-C) and KA2 (D-E) immunoreactivity in the monkey striatum. Te indicates immunoreactive terminals forming asymmetric synapses (arrowheads) with spines (SP) whereas asterisks label non-immunoreactive boutons. Note in D that gold particles (arrows) are occasionally found in the presynaptic grid of asymmetric synapses. In C the arrow indicates perisynaptic labelling a the edge of an asymmetric postsynaptic specialization. Scale bars: A: 0.25 μm; B: 0.5 μm (valid for D); C: 0.25 μm; E: 0.25 μm.

Fig. 4: (A,C) Relative proportion of intracellular versus membrane-bound immunogold labelling for GluR6/7 (A) and KA2 (C) in the monkey striatum. (B,D) Subsynaptic localization of membrane-bound immunogold labelling for GluR6/7 (B) and KA2 (D). Note that most gold particles are located extrasynaptically.

Fig. 5: Post-embedding immunogold localization of GluR6/7 (A-D) and KA2 (E-F) labelling in the monkey striatum. (A-B) Postsynaptic GluR6/7 immunoreactivity (arrows) in the main body of asymmetric axo-spinous synapses. (C,F) Presynaptic GluR6/7 (C) and KA2 (F) immunogold labelling (arrows) attached to the membrane of vesicular structures in terminals forming asymmetric synapses with spines (SP). (D,E) Pre (arrows) and postsynaptic (arrowhead) GluR6/7 (D) and KA2 (E) immunolabelling at asymmetric axo-spinous synapses. Note that the presynaptic labelling is aggregated in the presynaptic grid (arrows) whereas the postsynaptic labelling is associated with the postsynaptic density of the asymmetric specialization. Scale bars: A: 0.25 μm (valid for B-C,F); D: 0.25 μm (valid for E).

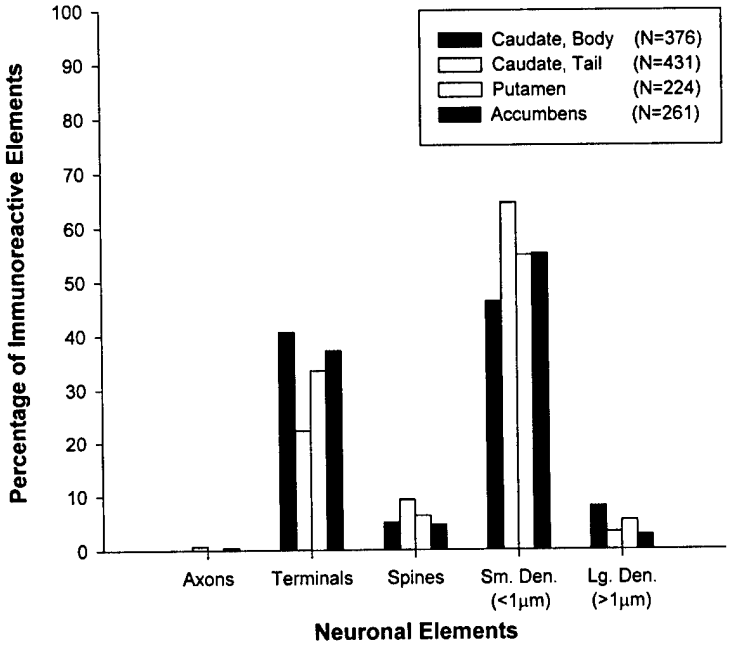
Fig. 6: Relative abundance of postembedding immunogold labelling for GluR6/7 (A,B) and KA2 (C,D) immunoreactivity in axon terminals (A,C) and spines (B,D).

Fig. 7: Subsynaptic localization of postembedding immunogold labelling for GluR6/7 (A) and KA2 (B) in axon terminals and spines. Note that the overall pattern of distribution resembles that found with the pre-embedding immunogold technique (see Fig. 4B,D).

Fig. 8: Localization of presynaptic GluR6/7 (A) and KA2 (B) immunogold labelling relative to asymmetric synaptic junctions. Note that both kainate receptor subunit immunoreactivity is randomly distributed relative to the synapses.

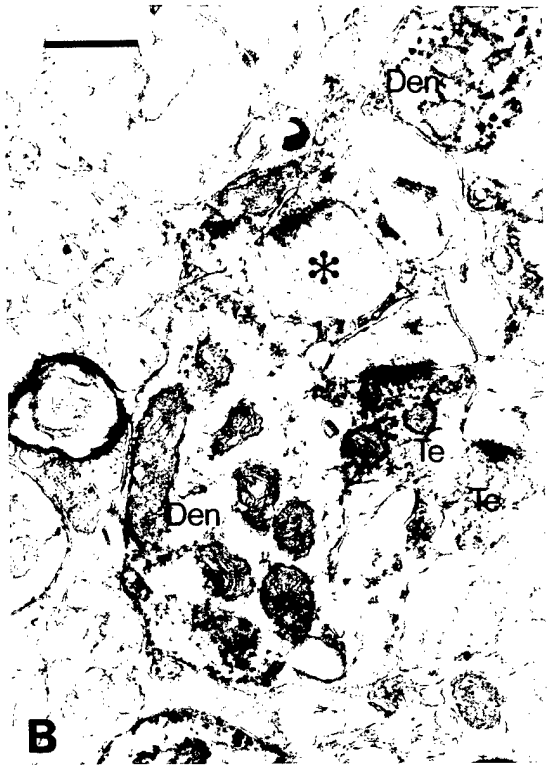
Fig. 9: Anterogradely labelled terminals immunoreactive for GluR6/7 (Te in A,C) or KA2 (Te in B,D) in the monkey putamen after BDA injections in the primary motor cortex (B,C) or the centromedian thalamic nucleus (A,D). All double labelled terminals formed asymmetric synapses (arrowheads) with spines (SP) or dendrites (Den). The asterisks indicate unlalelled boutons. A KA2-containing terminal (uTe) not labelled with BDA is also shown in B. Scale bars: A: 0.25 μm (valid for C); B: 0.25 μm (valid for D).

Fig. 10: Relative proportion of anterogradely labelled terminals that display GluR6/7 or KA2 immunoreactivity following thalamic or cortical injections of BDA.

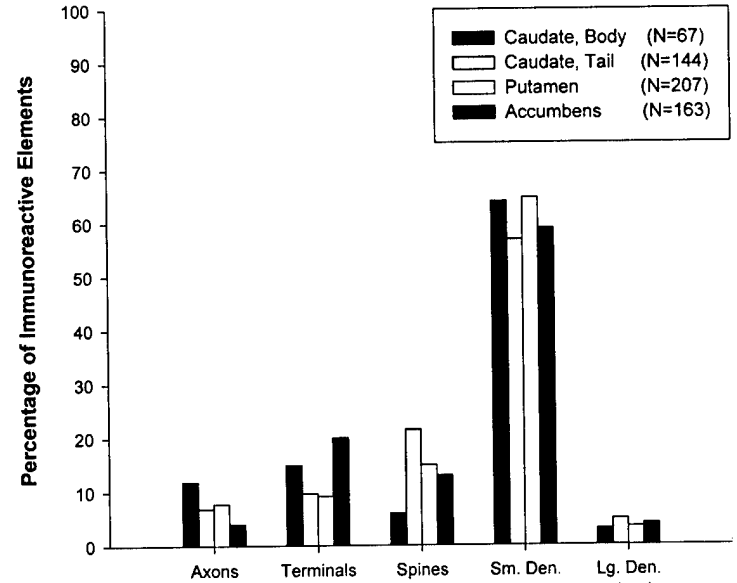


A

GLUR6/7 DISTRIBUTION

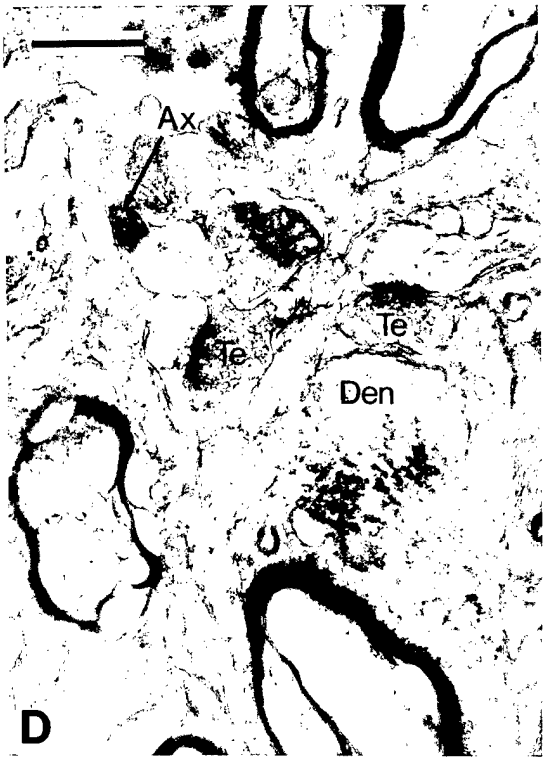


B



C

KA2 DISTRIBUTION



D

FIGURE 1

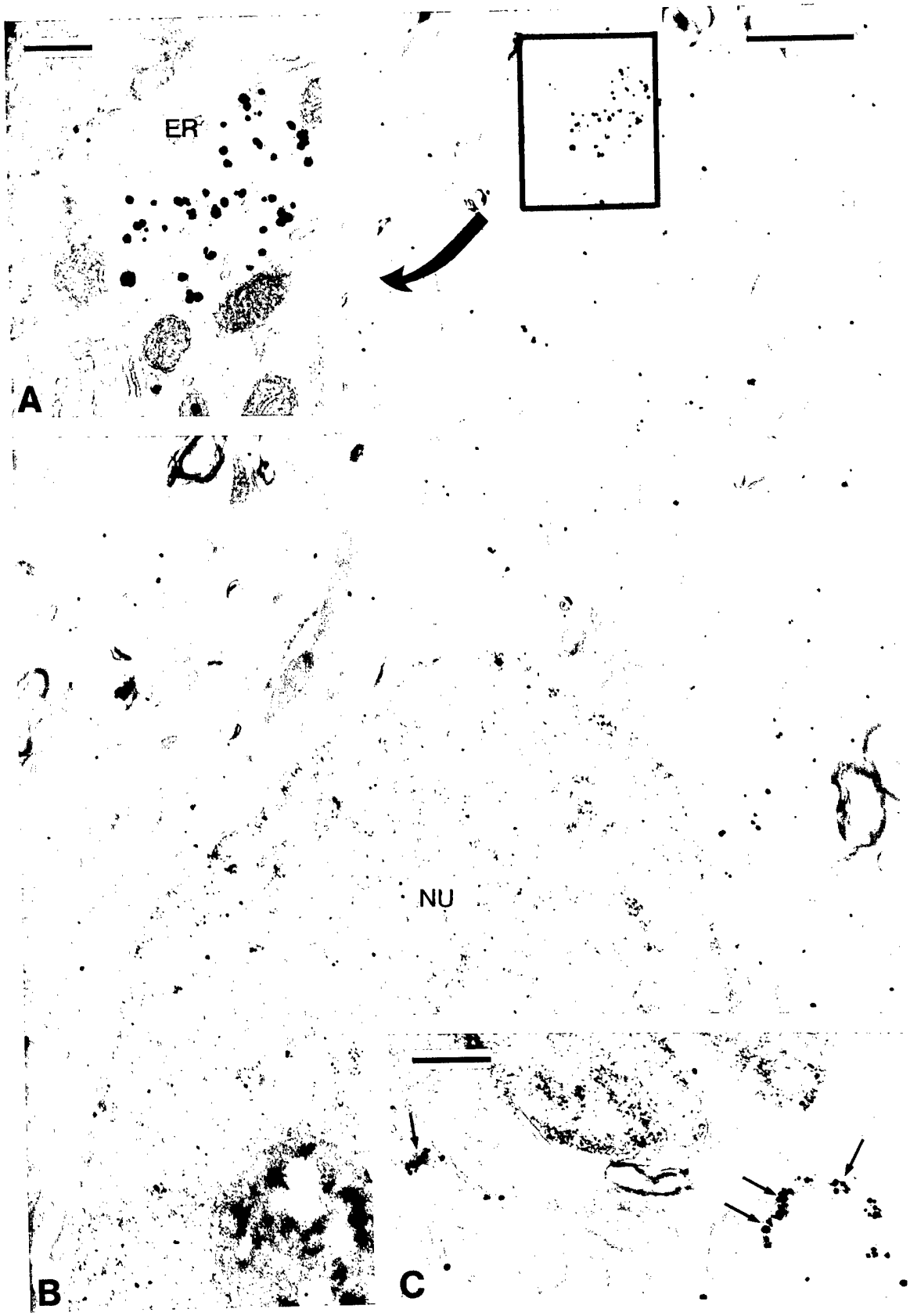


FIGURE 2

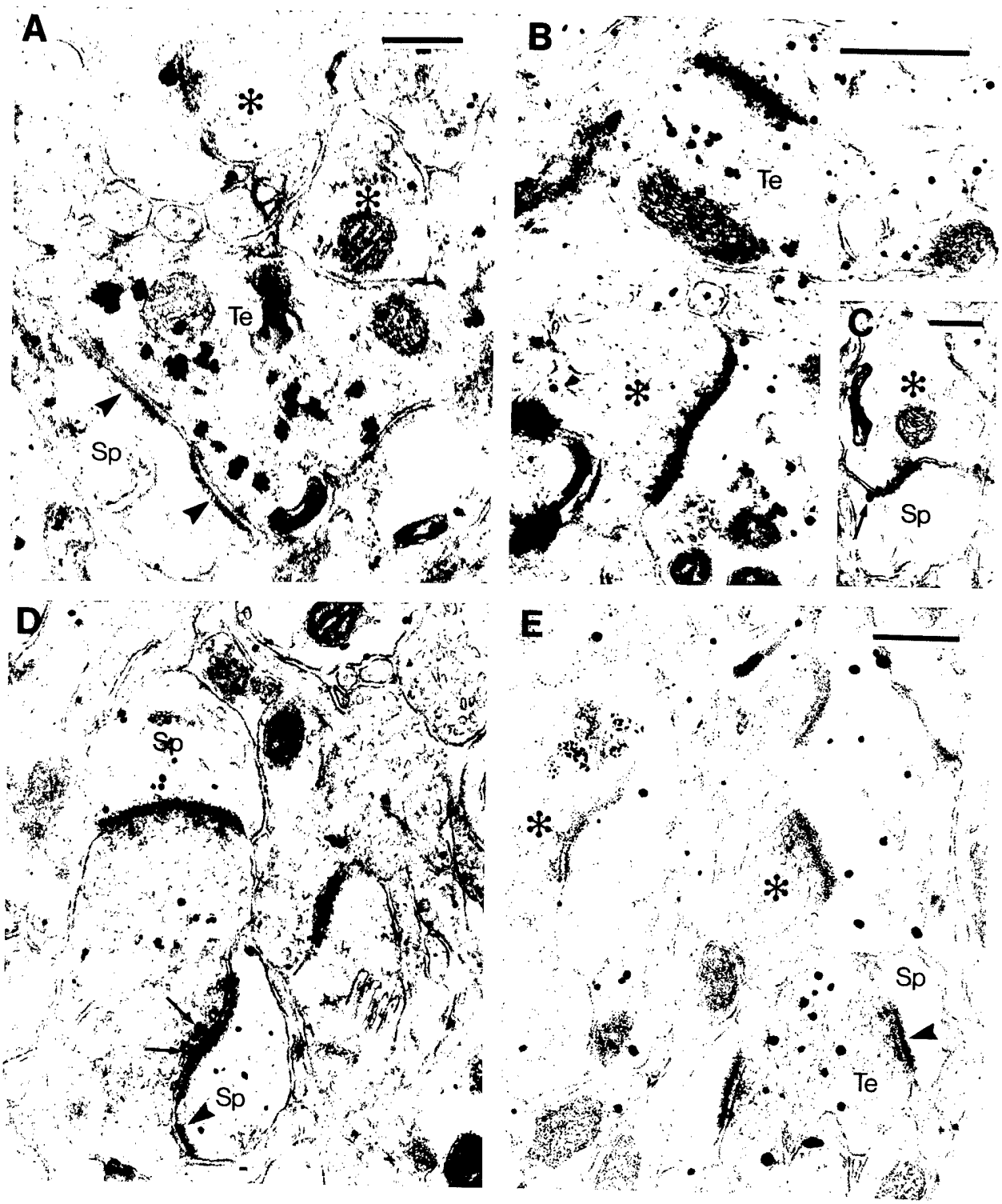
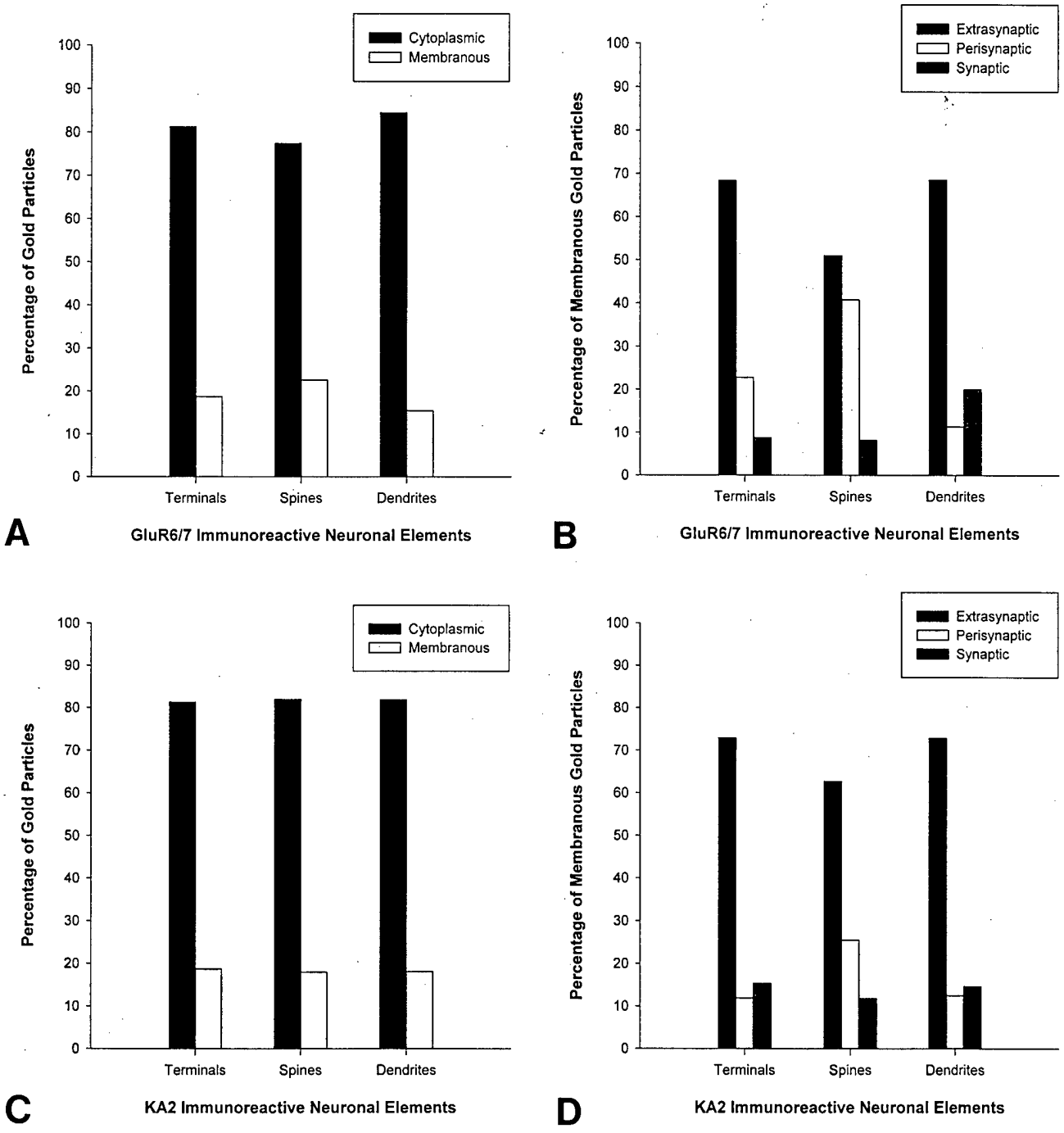


FIGURE 3

FIGURE 4


Cytoplasmic
Membranous
Percentage of Gold Particles
100
90
80
70
60
50
40
30
20
10
0
Terminals
Spines
Dendrites
A
GluR6/7 Immunoreactive Neuronal Elements
Extrasynaptic
Perisynaptic
Synaptic
Percentage of Membranous Gold Particles
B
GluR6/7 Immunoreactive Neuronal Elements
C
KA2 Immunoreactive Neuronal Elements
D
KA2 Immunoreactive Neuronal Elements

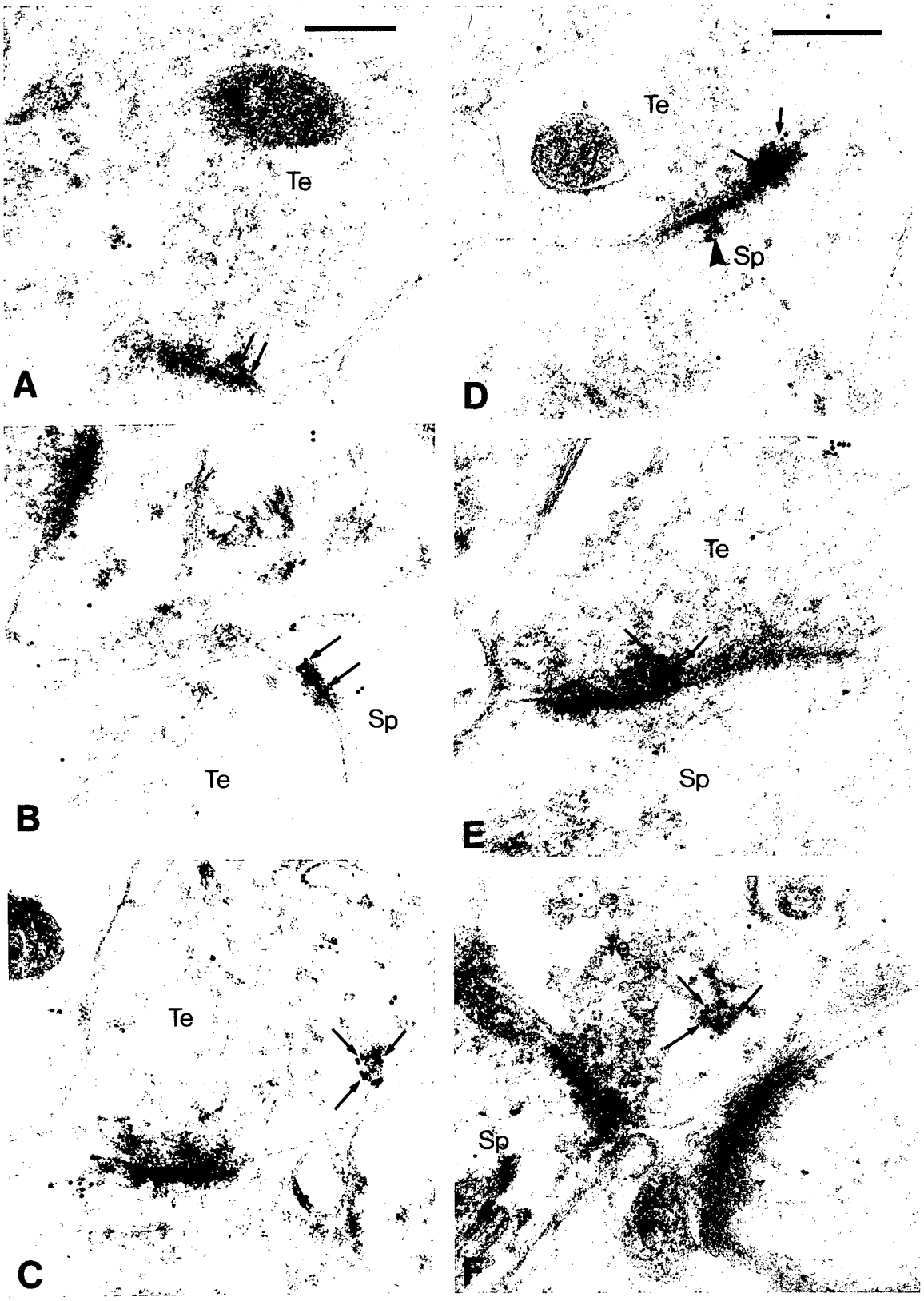
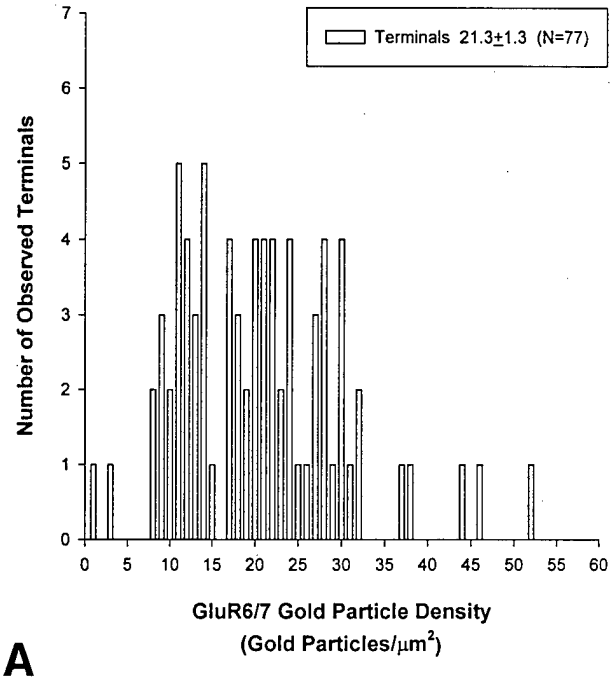
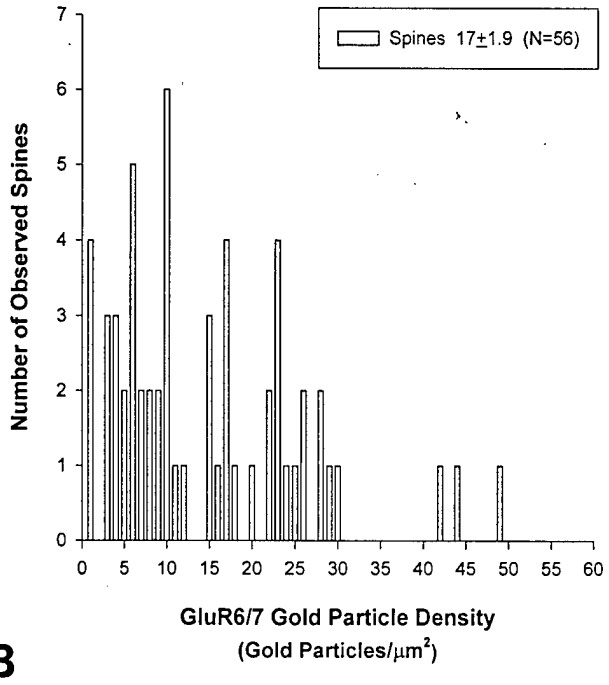
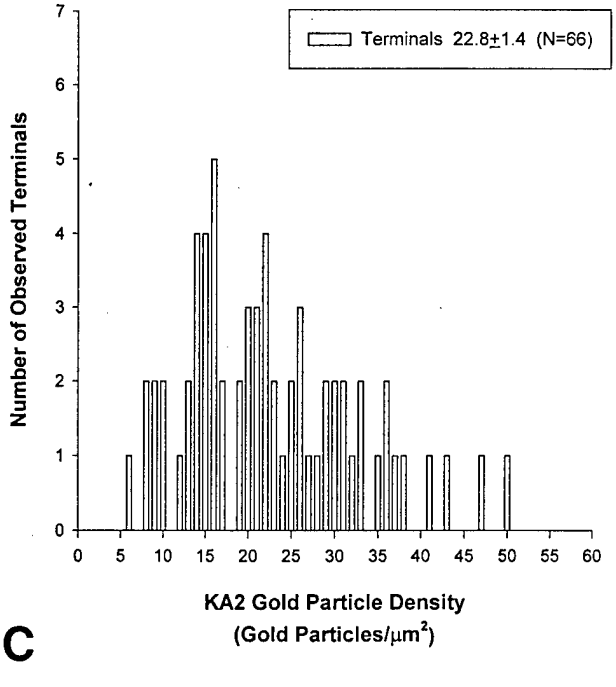


FIGURE 5


Terminals 21.3±1.3 (N=77)
Number of Observed Terminals
GluR6/7 Gold Particle Density
(Gold Particles/μm²)
A


Spines 17±1.9 (N=56)
Number of Observed Spines
GluR6/7 Gold Particle Density
(Gold Particles/μm²)
B


Terminals 22.8±1.4 (N=66)
Number of Observed Terminals
KA2 Gold Particle Density
(Gold Particles/μm²)
C

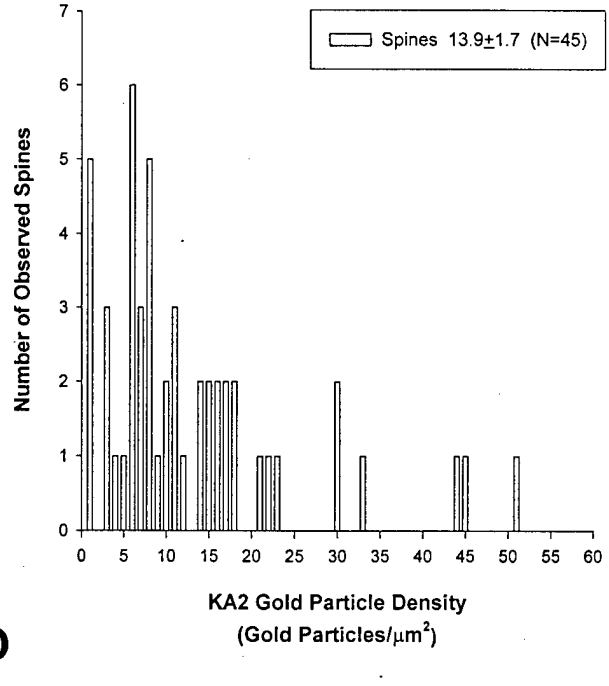
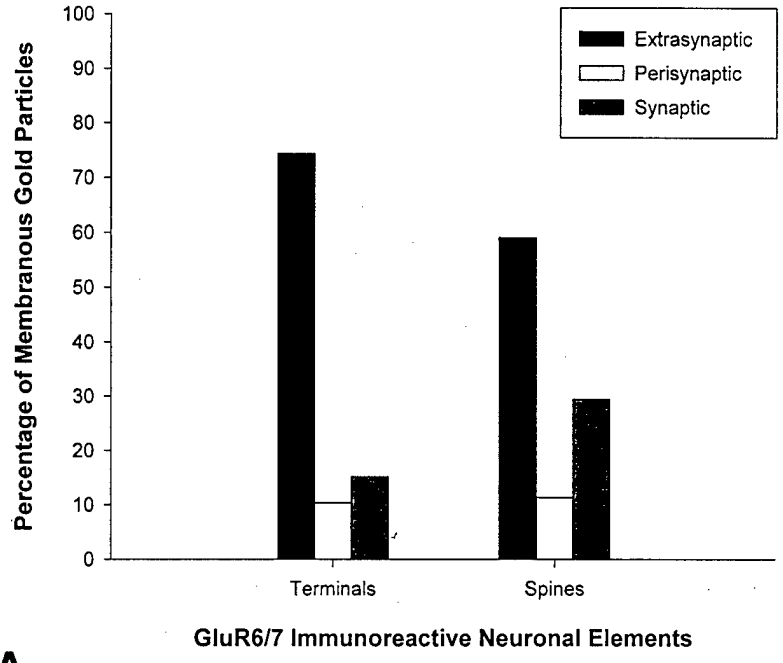
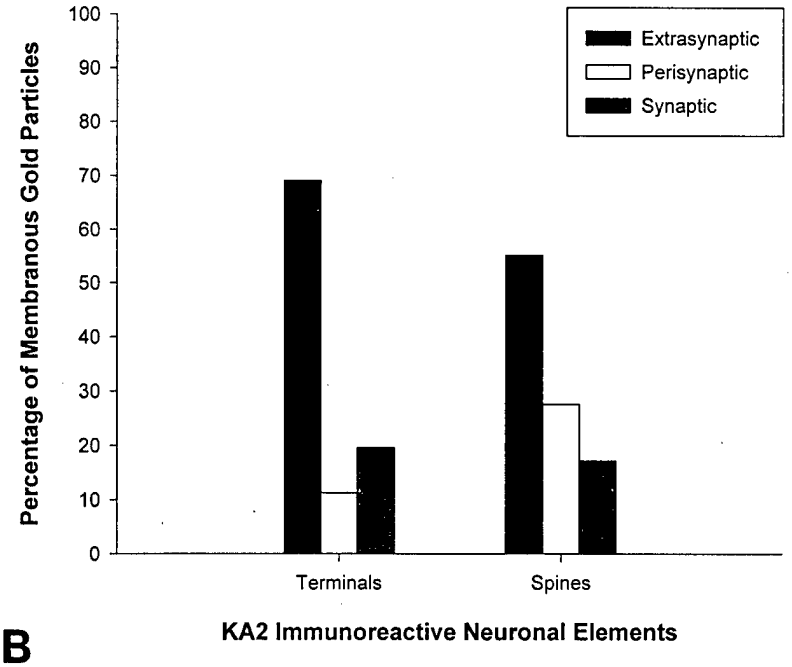

Spines 13.9±1.7 (N=45)
Number of Observed Spines
KA2 Gold Particle Density
(Gold Particles/μm²)
D

FIGURE 6

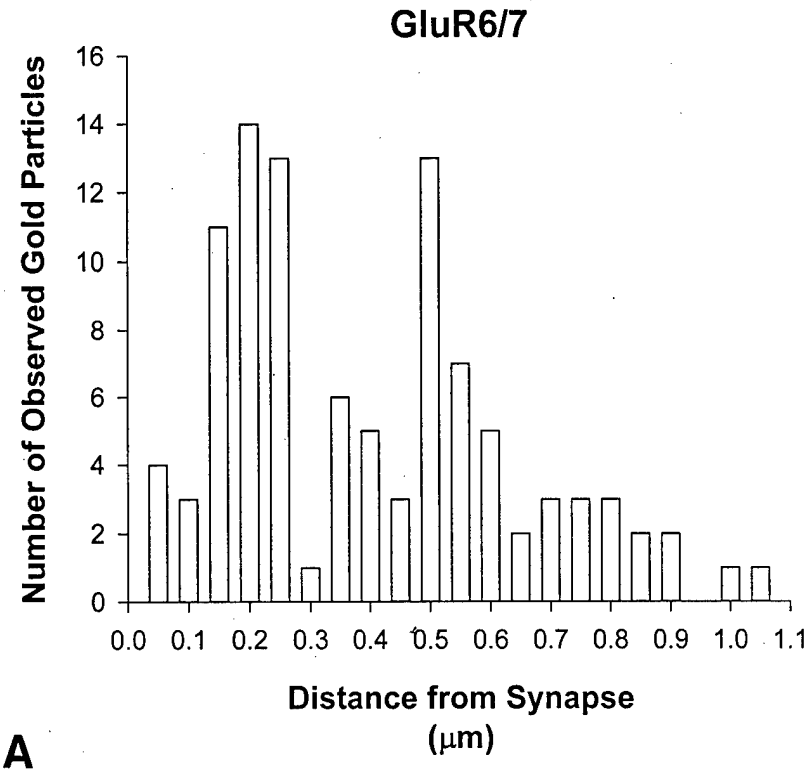

Percentage of Membranous Gold Particles
100
90
80
70
60
50
40
30
20
10
0
Extrasynaptic
Perisynaptic
Synaptic
Terminals
Spines
GluR6/7 Immunoreactive Neuronal Elements

A

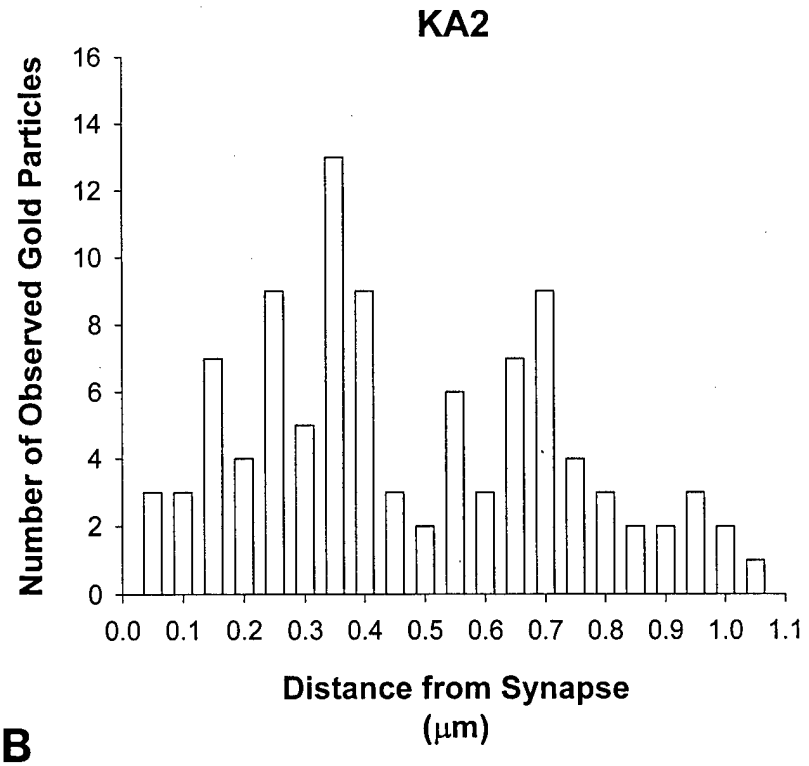

Percentage of Membranous Gold Particles
100
90
80
70
60
50
40
30
20
10
0
Extrasynaptic
Perisynaptic
Synaptic
Terminals
Spines
KA2 Immunoreactive Neuronal Elements

B

FIGURE 7


GluR6/7
Number of Observed Gold Particles
0
2
4
6
8
10
12
14
16
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1
Distance from Synapse
(μm)

A


KA2
Number of Observed Gold Particles
0
2
4
6
8
10
12
14
16
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1
Distance from Synapse
(μm)

B

FIGURE 8

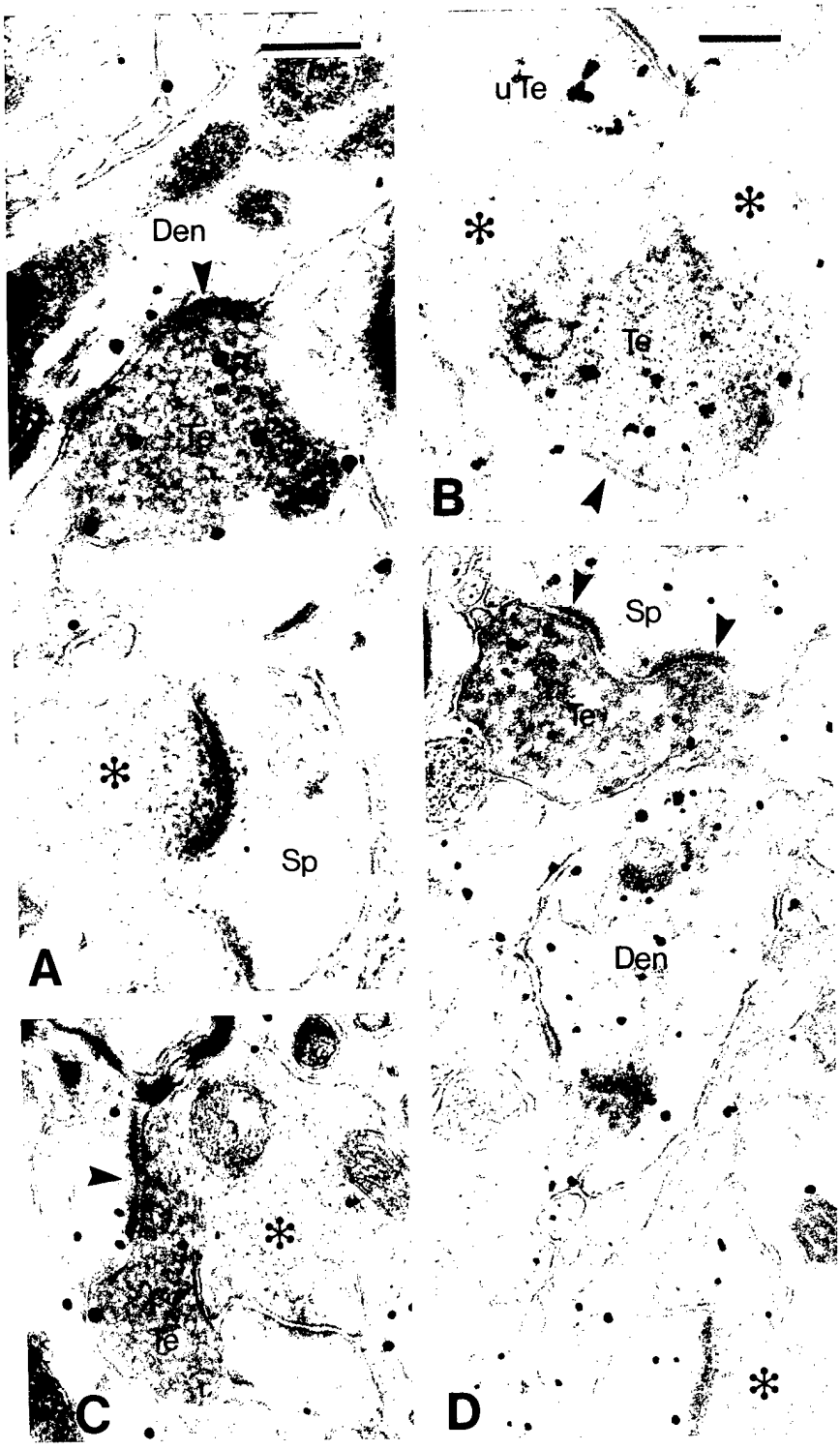
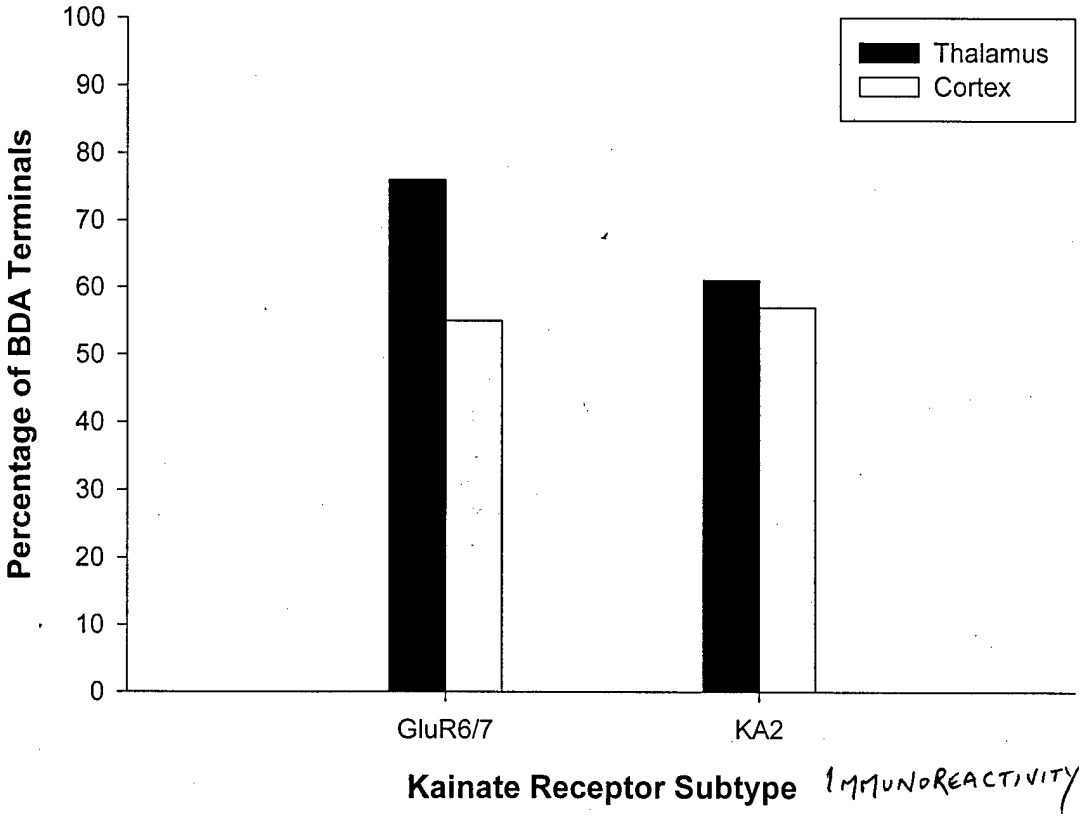


FIGURE 9

FIGURE 10


100
90
80
70
60
50
40
30
20
10
0
Percentage of BDA Terminals
Thalamus
Cortex
GluR6/7
KA2
Kainate Receptor Subtype
IMMUNOREACTIVITY

Enter the Author's Last Name:

Kieval

(You may narrow your search by entering multiple authors' last names separated by commas - e.g., Hodgkin, Huxley.)

Choose a Theme:

Any theme

Choose a Topic:

Any topic

Go

---

1 found.

Nov 8 PM - Poster Presentations

Day/Time	Abstract ID	Presentation Type	Location	Authors	1st Author 1st Affiliation	Title (Click to View Abstract)
Nov 8, 2000 3:00 PM - 4:00 PM	2528	Poster	Hall G-J	J.Z. Kieval[1*]; A. Charara[1,2]; J.F. Pare[1]; Y. Smith[1,2]	Div. Neuroscience Yerkes Primate Center, Emory University	SUBCELLULAR LOCALIZATION OF KAINATE RECEPTORS IN THE MONKEY STRIATUM

SUBCELLULAR LOCALIZATION OF KAINATE RECEPTORS IN THE MONKEY STRIATUM

J.Z. Kieval[1*]; A. Charara[1,2]; J.F. Pare[1]; Y. Smith[1,2]

1. Div. Neuroscience Yerkes Primate Center, 2. Dept. of Neurology, Emory University, Atlanta, GA, USA

Kainate receptor subunits are strongly expressed in glutamatergic terminals in the striatum. To better understand the function of these receptors and their possible control on neurotransmitter release, we have elucidated the subsynaptic localization of the GluR6/7 and KA2 subunits in the monkey striatum. At the LM level, both medium-sized projection neurons and a few large-sized neurons displayed GluR6/7 and KA2 immunoreactivity. At the EM level, immunoreactivity for both receptor subunits was found in perikarya, dendritic processes, and axon terminals in the body and tail of the caudate, putamen, and nucleus accumbens. No significant difference in the relative abundance of labeled elements was found in the various striatal regions. After injection of an anterograde tracer in the thalamus and cortex, kainate receptor immunoreactivity was seen in both populations of labeled terminals. Data obtained with pre-embedding immunogold was consistent with peroxidase results. Interestingly, a higher proportion of immunogold labeling was found intracellularly in all labeled elements. Of the gold particles bound to the plasma membrane, a majority were found extrasynaptically. Post-embedding immunogold data further supported these results. This technique demonstrated that a majority of the presynaptic kainate receptors are localized on vesicular membranes. Overall, our data show the localization of presynaptic kainate receptors intracellularly on vesicles in the monkey striatum. Such results might provide a better understanding of the mechanism by which kainate receptors mediate excitotoxic cell death in Huntington's disease.
Supported by: NIH grant RR00165 and a grant from the US Army

Close

IONOTROPIC AND METABOTROPIC GABA AND GLUTAMATE RECEPTORS IN THE PRIMATE BASAL GANGLIA

Yoland Smith [1,2], Ali Charara [1], Maryse Paquet [1], Jeremy Z. Kieval [1], Jesse E. Hanson[1], George W. Hubert [1], Masaaki Kuwajima [1] and Allan I. Levey [2]

[1] Division of Neuroscience, Yerkes Regional Primate Research Center, 954, Gatewood Rd NE, Atlanta, GA 30322, USA
[2] Department of Neurology, Emory University, Woodruff Memorial Building, Suite 6000, PO Drawer V, Atlanta, GA 30322, USA

Correspondence should be sent to: Yoland Smith, PhD
Yerkes Regional Primate Research Center
Emory University
954, Gatewood RD NE
Atlanta, GA 30322
USA

Tel: (404) 727 7519
Fax: (404) 727 3278
Email: yolands@rmy.emory.edu

ABSTRACT

The functions of glutamate and GABA in the CNS are mediated by ionotropic and metabotropic, G protein-coupled, receptors. Both receptor families are widely expressed in basal ganglia structures in primates and nonprimates. The recent development of highly specific antibodies and/or cDNA probes allowed to characterize better the cellular localization of various GABA and glutamate receptor subtypes in the primate basal ganglia. Furthermore, the use of high resolution immunogold techniques at the electron microscopic level led to major breakthroughs in our understanding of the subsynaptic and subcellular localization of these receptors in primates. In this review, we will provide a detailed account of the current knowledge of the localization of these receptors in the basal ganglia of humans and monkeys.

INTRODUCTION

Although the implication of "the basal ganglia" in the control of motor behaviors has long been known, the exact mechanisms by which these brain regions participate in motor control is still obscure and controversial. Furthermore, it is now clear that the functions of basal ganglia extend far beyond mere sensorimotor integration to include major cognitive and limbic components. The evidence that many neurodegenerative diseases of the basal ganglia often lead to major cognitive impairment accompanied by psychiatric problems strongly support the non-motor functions of these brain regions (Brown and Marsden, 1984, 1988; Marsden, 1984; Sano et al., 1989; Mayeux et al., 1990, 1992). Our knowledge of the anatomy and pathophysiology of primate basal ganglia has increased dramatically over the past twenty years due to the introduction of highly sensitive chemoanatomical methods, brain imaging techniques and the discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a chemical which selectively kills midbrain dopaminergic neurons in primates and induces Parkinson's disease (PD) (Davis et al., 1979; Langston et al., 1983). The MPTP model of PD is one of the best animal model of neurodegenerative diseases currently available. The use of this animal model has led to major breakthroughs in our understanding of the functional circuitry of the basal ganglia and served as the cornerstone for the development of novel surgical and pharmacological therapies for PD (see Starr, 1995; Blandini et al., 1996; Poewe and Granata, 1997; Vitek, 1997 for reviews).

The work that has been carried out in our laboratory over the past ten years has aimed at understanding various aspects of the connectivity and synaptic organization of the basal ganglia in non-human primates (Smith et al., 1998a,b). The recent development of highly sophisticated electron microscopic immunocytochemical approaches allowed us and others to better characterize the subsynaptic and subcellular localization of neurotransmitter receptors involved in mediating synaptic communication at various GABAergic and glutamatergic synapses in the primate basal ganglia (Paquet and Smith, 1996; Paquet et al., 1997; Waldvogel et al., 1998,

1999; Charara et al., 1999, 2000a; Hanson and Smith, 1999; Smith et al., 2000a). Unfortunately, due to problems inherent to postmortem tissue with the preservation of ultrastructural features, such studies cannot be carried out in human material. However, due to similarities in the subcortical organization of basal ganglia structures in human and non-human primates, there is a high likelihood that our findings in monkeys can be extrapolated to humans. In this review, we will present some of our most recent data on the subsynaptic localization of metabotropic glutamate receptors and GABA-B receptors in the monkey basal ganglia. We will also give a brief overview of the current knowledge of the localization of various subtypes of glutamate and GABA receptors in the human basal ganglia largely based on data gathered by autoradiographic binding studies, *in situ* hybridization method, light microscopic immunocytochemical method and PET imaging technique. Finally, we will examine the possibility of using novel drug therapies directed towards specific subtypes of G protein-coupled glutamate and GABA receptors to treat Parkinson's disease.

Because of the scope of the paper, this review will mostly cover data gathered in monkeys and humans. The reader is referred to recent extensive reviews and compendia related to basal ganglia research for a more extensive coverage of literature (Joel and Weiner, 1994, 1997; Percheron et al., 1994; Parent and Hazrati, 1995; Chesselet and Delfs, 1996; Gerfen and Wilson, 1996; Ohye et al., 1996; Levy et al., 1997; Smith et al., 1998a, b; Wilson, 1998).

BASAL GANGLIA CIRCUITRY

Striatal Afferents

In primates, the basal ganglia are comprised of five tightly interconnected subcortical structures involved in the integration and processing of sensorimotor, cognitive and limbic information. The main entrance of cortical information to the basal ganglia circuitry is the striatum which is comprised of the caudate nucleus (CD), putamen (PUT) and nucleus

accumbens. The glutamatergic corticostriatal projection is highly topographic and imposes a functional compartmentation of striatal regions. The post-commissural putamen receives inputs from the primary motor and somatosensory cortices as well as pre-motor and supplementary motor areas whereas the pre-commissural putamen and the caudate nucleus are the main targets of associative cortical regions. On the other hand, the bulk of cortical afferents to the nucleus accumbens (Acc) arise from limbic cortices, amygdala and hippocampus (Heimer et al., 1995). Another level of striatal compartmentation is the patch/matrix organization. This concept, which originally relied upon the heterogeneous distribution of acetylcholinesterase is now considered to be a basic framework of striatal architecture. Most neurotransmitters and neuropeptides as well as major striatal afferent projections and striatal output neurons display a preferential distribution for the patch or the matrix compartment (Graybiel, 1990).

Another major glutamatergic input to the striatum arises from the caudal intralaminar thalamic nuclei, namely the centromedian (CM) and parafascicular (Pf) nuclear complex (Smith and Parent, 1986; Sadikot et al., 1992a,b; Parent and Hazrati, 1995). Projections from the CM terminate preferentially in the sensorimotor striatal territory whereas inputs from Pf innervate the associative and limbic striatal regions (Sadikot et al., 1992b). Finally, the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) are the two main sources of dopamine to the dorsal and ventral striatum, respectively. Additional sources of innervation of the striatum include the external globus pallidus (GPe), the subthalamic nucleus (STN), the dorsal raphe and the pedunculopontine nucleus (TPP) (Smith and Parent, 1986) (Fig.1).

The main targets of striatal afferents are the GABAergic medium sized spiny projection neurons which account for more than 90% of the total neuronal population of the striatum (Smith and Bolam, 1990). The glutamatergic inputs from the cortex terminate almost exclusively on the heads of dendritic spines whereas the thalamic afferents from CM/Pf preferentially innervate dendritic trunks (Sadikot et al., 1992a, Smith et al., 1994). Dopamine and cortical inputs often converge at the level of individual spines, which supports the tight functional interaction between dopamine and glutamate in mediating proper basal ganglia functions (Smith et al., 1994). In

addition to projection neurons, the striatum is also endowed with various populations of aspiny interneurons recognized by their size and differential content in neurotransmitter, neuropeptides and calcium binding proteins. Four main classes of interneurons have been recognized in the primate striatum: (1) the cholinergic neurons, (2) the parvalbumin-containing neurons, which co-express GABA, (3) the somatostatin-containing neurons which also contain neuropeptide Y and nitric oxide synthase and (4) the calretinin-containing neurons. Albeit less massively innervated than spiny neurons, interneurons also receive direct cortical, thalamic and nigral inputs (Kawaguchi et al., 1995; Sidibé et al., 1999; Bolam et al., 2000).

Direct and Indirect Striatofugal Pathways

Once integrated and processed at the striatal level, the information is conveyed to the basal ganglia output structures, the internal globus pallidus (GPi) and the substantia nigra pars reticulata (SNr), via two pathways: (1) The direct pathway, which arises from a subset of striatal projection neurons enriched in substance P/dynorphin and D1 dopamine receptors, terminates directly in GPi and SNr or (2) the indirect pathways, which originate from striatal neurons enriched in enkephalin and D2 dopamine receptors, terminate in GPe (Albin et al., 1989; Bergman et al., 1990; Gerfen et al., 1990). In turn, GPe neurons provide GABAergic inputs to the STN which relays signals to the basal ganglia output nuclei. Imbalance in the activity of these two pathways, in favor of the indirect pathway, underlies some of the motor deficits in Parkinson's disease (DeLong, 1990; Wichmann and DeLong, 1996). The model of direct and indirect pathways as originally introduced was, by necessity, a simplification and only included the major projections of sub-nuclei of the basal ganglia (Albin et al., 1989; Bergman et al., 1990). However, since its introduction there have been many developments in our knowledge and understanding of the anatomical and synaptic organisation of the basal ganglia that have led to reconsideration and updates of some aspects of the model. One of the most important new finding regarding the anatomical organization of the basal ganglia is the demonstration of

multiple indirect pathways of information flow through the basal ganglia. In addition to the classical indirect pathway through the GPe and the STN, it is now well established that the GPe gives rise to GABAergic projections that terminate in basal ganglia output structures (GPi, SNr), the reticular nucleus of the thalamus and the striatum (see Parent and Hazrati, 1995; Smith et al., 1998a,b for reviews) (Fig. 1). Even if the exact functions of these connections remain unknown, it should be kept in mind that the circuitry of the basal ganglia as outlined in the original model of "direct and indirect" pathways is likely to be more complex than previously thought (Smith et al., 1998a). It is noteworthy that molecular and anatomical data showing: (1) a higher degree of co-localization of D1 and D2 dopamine receptors in striatal projection neurons and (2) a higher degree of collateralization of individual "direct" striatofugal neurons recently challenged the concept of direct and indirect pathways (see Gerfen and Wilson, 1996 for a review). Although these findings do not rule out the segregation of striatofugal neurons, they must be kept in mind while considering the functional significance of the direct and indirect striatofugal pathways in normal and pathological conditions.

Basal Ganglia Outflow

Once the information has reached the GPi and SNr, it is conveyed to various thalamic and brainstem nuclei which project to motor and pre-motor cortical areas or to lower brainstem regions. Although both the GPi and SNr project to the ventral anterior/ventral lateral thalamic complex (VA/VL), the nigral and pallidal afferents largely terminate in different subdivisions of the VA/VL nuclei in primates (Ilinsky et al., 1993). Other targets of SNr neurons include the brainstem pedunculopontine nucleus (TPP), the superior colliculus and the medullary reticular formation (Fig. 1). The nigrocollicular fibers, which terminate mainly onto tectospinal neurones in the intermediate layer of the superior colliculus, play a critical role in the control of visual saccades. At thalamic level, inputs from the medial part of the SNr terminate mostly in the medial magnocellular division of the VA (VAmc) and the mediodorsal nucleus (MDmc) which,

in turn, innervate anterior regions of the frontal lobe including the principal sulcus (Walker's area 46) and the orbital cortex (Walker's area 11) in monkeys (Ilinsky et al., 1985). On the other hand, neurones in the lateral part of the SNr project preferentially to the lateral posterior region of the VAmc and to different parts of the MD mostly related to posterior regions of the frontal lobe including the frontal eye field and areas of the premotor cortex (see Sidibé et al., 1997 for a review). Another thalamic target of SNr neurons is the caudal intralaminar Pf, which provides a massive feedback projection to the CD (Ilinsky et al., 1985; Smith et al., 2000b).

In addition to the VA/VL and caudal intralaminar thalamic nuclei, the lateral habenular nucleus and the TPP also receive significant inputs from GPi. Efferents from the sensorimotor GPi remain largely segregated from the associative and limbic projections at the level of the thalamus whereas they partly overlap in the TPP (Shink et al., 1997; Sidibé et al., 1997). On the other hand, limbic and associative pallidal projections innervate common nuclei in the thalamus and TPP. In squirrel monkeys, the sensorimotor GPi outputs are directed towards the posterior VL (VLp), whereas the associative and limbic GPi preferentially innervate the parvocellular ventral anterior (VApc) and the dorsal VL (VLd). The ventromedial nucleus receives inputs from the limbic GPi only (Sidibé et al., 1997). These findings, therefore, suggest that some associative and limbic cortical information, which is largely processed in segregated corticostriatopallidal channels, converge at common thalamic nuclei in monkeys (Sidibé et al., 1997). The basal ganglia influences are then conveyed to the cerebral cortex via the VA/VL nuclei. Although it has long been thought that the sensorimotor information from the GPi was conveyed exclusively to the supplementary motor area (SMA), recent anatomical and physiological data in macaques demonstrate that the information from the GPi may also be sent to the primary motor cortex (M1) and the pre-motor (PM) cortical area (Rouillier et al., 1994; Hoover and Strick, 1999). Retrograde transneuronal virus studies showed that different populations of GPi neurones project to SMA, M1 and PM (Middleton and Strick, 2000).

Most pallidal neurons which project to thalamic relay nuclei send axon collaterals to the caudal intralaminar nuclei where they are distributed according to a specific pattern of functional

organization. Pallidal axons arising from the sensorimotor GPi terminate exclusively in CM where they form synapses with thalamostriatal neurons projecting back to the sensorimotor territory of the striatum (Smith and Sidibé, 1999). In contrast, associative inputs from the caudate-receiving territory of GPi terminate massively in a dorsolateral extension of PF (PFdl) which, surprisingly, does not project back to the caudate nucleus but rather preferentially innervates the pre-commissural region of the putamen. Finally, the limbic GPi selectively innervates the rostrodorsal part of PF which gives rise to the thalamo-accumbens projection (Sidibé et al., 1997; Smith et al., 1998b; Smith and Sidibé, 1999). Therefore, it appears that the CM/PF is part of closed and open functional loops with the striatopallidal complex (Smith and Sidibé, 1999). Neurons in PF that project to the caudate nucleus do not receive inputs from any functional regions of GPi, but receive substantial innervation from the SNr (Smith et al., 2000b).

In monkeys, more than 80% of GPi neurones that project to the VA/VL send axon collaterals to the TPP (Parent and Hazrati, 1995). In contrast to the thalamus that conveys the basal ganglia information to the cerebral cortex, the TPP gives rise to descending projections to the pons, medulla and spinal cord as well as prominent ascending projections to the different structures of the basal ganglia, the thalamus and the basal forebrain (Inglis and Winn, 1995; Rye, 1997). The pallidotegmental projection may thus be a route by which information can escape from the basal ganglia-thalamocortical circuitry and reach lower motor and autonomic centers.

GLUTAMATE AND GABA RECEPTOR FAMILIES IN THE CNS

Glutamate and GABA receptors are categorized in two main groups based on their structure and mechanisms of actions. The ionotropic receptors are ligand-gated ion channels which mediate fast synaptic transmission whereas the metabotropic receptors are coupled to G proteins and initiate intracellular signaling cascades.

Ionotropic and Metabotropic Glutamate Receptors

Two main subtypes of ionotropic glutamate receptors have been identified: the N-methyl-D-aspartate (NMDA) receptors and the alpha-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA)/kainate receptors. Numerous subunits and variants constituting the different types of ionotropic glutamate receptors have now been cloned and sequenced. Various factors, including the subunit composition and the relative abundance of these subunits influence the biophysical properties of these receptors. The NMDA receptors consist of NMDAR1 and NMDAR2 (a,b,c,d,e splice variants) subunits whereas the AMPA receptors are made up of the GluR1-4 subunits. Finally, heteromeric combinations of the high-affinity kainate binding subunits (GluR5-7; KA1-2) form the kainate receptors (Gasic and Hollmann, 1992; Hollmann and Heineman, 1994; Westbrook, 1994). In general, activation of AMPA and kainate receptors is responsible for primary events in fast glutamatergic transmission since NMDA receptors only become fully activated by glutamate secondarily when their Mg^{+2} block is relieved by depolarization.

The metabotropic glutamate receptor family includes eight different subtypes pooled into three major groups based on their sequence homology, pharmacological properties and transduction mechanisms. The group I mGluRs, which include the splice variants of mGluR1 (a,b,c,d) and mGluR5 (a,b), are positively coupled via Gq to phospholipase C and PI hydrolysis. Activation of these receptors, which are usually found postsynaptically, generally leads to slow depolarization, though presynaptic group I mGluRs were also found in some brain regions (Nakanishi, 1994; Pin and Duvoisin, 1995; Conn and Pin, 1997). Group II mGluRs (mGluR2,3) are negatively coupled via Gi/Go to adenylyl cyclase and inhibit the formation of cyclic AMP following exposure to forskolin or activation of an intrinsic Gs-coupled receptor. Similarly, group III mGluRs (mGluR4,6,7,8) inhibit adenylyl cyclase via a pertussis toxin sensitive G-protein. Group II and group III mGluRs are generally found presynaptically where they act as auto- or heteroreceptors to modulate the release of glutamate or other neurotransmitters. The

three groups of mGluRs can be further differentiated pharmacologically by their selective sensitivity to specific agonists (Nakanishi, 1994; Conn and Pin, 1997; Schoepp et al., 1999). However, selective compounds for specific subtypes of receptors in the same group are still missing, except for the recent development of specific antagonists for mGluR1 and mGluR5 (see Schoepp et al., 1999 for a review).

Three Major Groups of GABA Receptors

The GABA receptors are pooled into three groups. The GABA-A receptors, which are ligand-gated chloride channels, mediate fast inhibitory transmission in the CNS. These receptors are pentameric glycoproteins made up of various subunits which, to date, can be categorized into seven groups based on sequence homology (6α, 4β, 4γ, 1δ, 1ε, 1π, 1θ) (McDonald and Olsen, 1994; Bonnert et al., 1999; Mehta and Ticku, 1999). The combinational assembly of these subunits (and splice variants of several of them) into a pentameric structure results in diverse receptor subtypes. In vivo, fully functional GABA-A receptors are generally made up of a combination of α, β and γ2 subunits. About 80% of all GABA-A receptors in the CNS are sensitive to benzodiazepines (BZ) and contain the classical BZ binding sites (Möhler et al., 1997; Upton and Blackburn, 1997; Mehta and Ticku, 1999). The γ2 subunit is essential to convey BZ sensitivity to GABA-A receptors which is consistent with the widespread distribution of this subunit in the brain. This BZ sensitivity has been instrumental for the development of various ligands to map the distribution of GABA-A receptor binding sites in the human CNS (see below). Another particular feature of GABA-A receptors is their sensitivity for the highly specific agonist and antagonist, muscimol and bicuculline.

Another major subtype of GABA receptors in the CNS is the GABA-B receptors which were introduced in the early '80s as a novel type of bicuculline-insensitive Cl^--independent GABA receptors (Hill and Bowery, 1981). GABA-B receptors, which are selectively activated by baclofen and insensitive to bicuculline (Bowery et al., 1980; Hill and Bowery, 1981), belong

to the family of seven transmembrane domain receptors and are coupled to Ca^{2+} and K^+ channels via G proteins and second messenger systems, e.g. inhibiting adenylate cyclase (Bormann, 1997; Bowery, 1997; Deisz, 1997). GABA-B receptors generate the late inhibitory postsynaptic potentials that are important for the fine tuning of inhibitory neurotransmission (Bettler et al., 1998). During the past few years, an impressive amount of work has been devoted to the mechanisms of GABA-B action in the CNS (see reviews by Kerr and Ong, 1995; Misgeld et al., 1995; Bowery, 1997; Deisz, 1997). The development of potent GABA-B antagonists (Bittiger et al., 1993; Olpe et al., 1990; Ong et al., 1999; Froestl et al., 1999) has greatly facilitated the investigation of the various facets of GABA-B receptors. Recent cloning of GABA-B receptor subtypes revealed extended similarity with metabotropic glutamate receptors. So far, two GABA-B receptor subtypes have been identified, GABA-BR1 and GABA-BR2 which assemble into heterodimers to form a functional GABA-B receptor (Bettler et al., 1998; Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999; Makoff, 1999; Martin et al., 1999).

Finally, the last group of GABA receptors are the GABA-C receptors which, like GABA-A receptors, are members of the ligand-gated ion-channel superfamily receptors permeable to chloride ions. However, GABA-C receptors stand as a separate class of receptors due to their lack of sensitivity to bicuculine and baclofen (Johnston, 1997). Another feature that characterizes GABA-C receptors is their lack of sensitivity to benzodiazepines and barbiturates. Furthermore, these receptors are activated at lower concentrations of GABA and are less liable to desensitization than most GABA-A receptors. GABA-C receptors are made up of ρ subunits which were cloned in the early '90s from a human retinal library. To date, three ρ subunit cDNAs have been characterized, but very little is known about their functions and distribution in the primate brain (Johnston, 1997). Although originally thought to be expressed exclusively in the retina, recent *in situ* hybridization studies revealed a much broader distribution of these receptor subunits in the rodent and human brain (Enz et al., 1995; Wegelius et al., 1998; Enz and Cutting, 1999).

TECHNICAL DEVELOPMENTS FOR THE LOCALIZATION OF NEUROTRANSMITTER RECEPTORS

The cloning techniques combined with the development of sensitive high resolution electron microscopic immunocytochemical methods led to major breakthroughs in our current knowledge of receptor localization in the CNS. Although autoradiographic ligand binding method has long been a major tool used to visualize receptor binding sites in the brain, the lack of resolution of this approach significantly hampers the interpretation of the exact neuronal localization of receptor subtypes. A better resolution can be obtained using the techniques of light and electron microscopic immunocytochemistry and *in situ* hybridization. However, due to limitations inherent to postmortem tissue in the preservation of membranes and glycoprotein antigenicity, the immunocytochemical detection of receptors at the electron microscopic level in human brain is limited, which makes the ligand binding methods still the most commonly used tool to study receptor distribution in postmortmem human brain tissue (see below).

The electron microscopic immunogold methods provide a way by which the exact localization and relative abundance of receptors in relation to specific release sites of neurotransmitters can be studied. In our laboratory, we use two different immunogold methods to study glutamate and GABA receptors in the monkey basal ganglia, namely the pre-embedding silver-intensified immunogold method and the freeze-substitution post-embedding immunogold technique. These approaches combined with the regular immunoperoxidase method provide complementary information which help characterize the precise localization of a particular receptor in the brain. The main advantage of the immunogold methods over the immunoperoxidase technique is the higher level of spatial resolution of gold particles in comparison to the amorphous diaminobenzidine (DAB) reaction product. However, the immunoperoxidase approach still remains the most sensitive technique to detect low level of receptor proteins. In the pre-embedding immunogold method, a secondary antibody conjugated

to 1.4 nm gold particles, rather than the peroxidase complexes, is used to localize the antigenic sites. The size of the gold particles is, then, increased by silver intensification which results in 30-50 nm electron-dense particles. The main problem with this approach is the poor penetration of gold-conjugated antibodies which limits the electron microscopic analysis to the most superficial part of tissue sections and significantly hampers the interpretation of negative data. For that reason, the post-embedding immunogold technique is definitely the only reliable approach to quantify and unequivocally compare receptor densities associated with different synapses on the same section. In order to maintain the antigenicity of GABA and glutamate receptors after embedding, it is necessary to use the technique of fast freezing followed by low temperature dehydration (a process named freeze-substitution) and low temperature embedding of fixed brain tissue in non-polar resin (Baude et al., 1993, 1995; Lujan et al., 1996; Nusser et al., 1994; 1995; 1997; 1998; Matsubara et al., 1996; Bernard et al., 1997; Ottersen and Landsend 1997; Bernard and Bolam, 1998; Clarke and Bolam, 1998; Nusser, 1999).

GLUTAMATE AND GABA RECEPTORS IN THE STRIATUM

Ionotropic Glutamate Receptors

AMPA Receptors

The localization of AMPA receptors in the human striatum has been studied in normal and pathological conditions by means of autoradiographic ligand binding (Dure et al., 1991, 1992; Lee and Choi, 1992; Ball et al., 1994; Noga et al., 1997; Healy et al., 1998; Blue et al., 1999), *in situ* hybridization (Bernard et al., 1996; Tomiyama et al., 1997; Healy et al., 1998) and light microscopic immunohistochemistry (Meng et al., 1997; Cicchetti et al., 1999). Overall, the whole human striatum is quite enriched in AMPA receptor binding sites but a slightly higher density is found in the matrix compartment (Dure et al., 1992). Analysis of striatal AMPA

binding sites in various pathological conditions revealed: (1) either no significant differences (Healy et al., 1998) or increases (Noga et al., 1997) in AMPA binding sites in schizophrenics, (2) significant reductions in AMPA receptor bindings in the putamen of girls with Rett syndrome (Blue et al., 1999) and (3) significant decreases of AMPA binding sites in the caudate nucleus of Huntington's patients (Dure et al., 1991). More recent studies using *in situ* hybridization and immunohistochemical methods revealed that the GluR1,2,3 subunits are widely expressed in both projection neurons and large interneurons in the human striatum whereas GluR4 is confined to a small population of large- and medium-sized neurons (Bernard et al., 1996; Meng et al., 1997; Tomiyama et al., 1997). Subpopulations of large and medium CR-containing interneurons are endowed with GluR1, GluR2 and GluR4 AMPA receptor subunits (Cicchetti et al., 1999). No significant changes in GluR1-4 mRNA expression was found in the striatum of parkinsonian patients (Bernard et al., 1996).

As in humans, GluR1,2,4 immunoreactivities are found in medium-sized projection neurons in both the caudate nucleus and putamen of rhesus monkeys (Martin et al., 1993). In contrast, large cholinergic interneurons, which partly co-localize with CR in humans (Cichetti et al., 1998), express GluR4 immunoreactivity but are devoid of GluR1 and GluR2/3 labeling (Martin et al., 1993). GluR1, but not GluR2/3 or GluR4 immunoreactivity, is more intense in the ventral striatum than the dorsal striatum. In the caudate nucleus, GluR1 is preferentially expressed in medium sized spiny neurons in patches whereas the matrix contains large GluR4-containing cholinergic interneurons (Martin et al., 1993). Recent data showed an upregulation of the AMPA GluR1 subunit in the striatal patch compartment of MPTP-treated parkinsonian monkeys (Betarbet et al., 2000). Most intrinsic dopaminergic neurons in the monkey striatum, which increase substantially in number after MPTP treatment, express GluR1, but not GluR2/3 immunoreactivity (Betarbet and Greenamyre, 1999).

At the electron microscopic level, the GluR1 subunit is enriched in dendritic spines (Martin et al., 1993), which is consistent with recent immunogold data showing that the bulk of

AMPA receptor subunit immunoreactivity is confined to asymmetric axo-spinous synapses in the rat striatum (Bernard et al., 1997).

Kainate Receptors

Due to the lack of specific markers that could differentiate kainate from AMPA receptor binding sites, very little is known about the localization of kainate receptors in the human striatum. Using *in situ* hybridization approach, Bernard et al. (1996) recently showed that the GluR6, GluR7 and KA2 receptor subunits are detected in about 50-60% of striatal medium-sized neurons whereas the KA1 labeling is restricted to 20-30% of these neurons. Less than 2% of striatal neurons express the GluR5 subunit mRNA (Bernard et al., 1996).

We recently carried out a detailed analysis of the subsynaptic localization of kainate receptor subunit immunoreactivity in the monkey striatum using antibodies raised against the GluR6/7 and KA2 kainate receptor subunits (Fig. 2). One of the major finding of this study was that kainate receptor subunits are expressed presynaptically in glutamatergic axon terminals forming asymmetric axo-spinous and axo-dendritic synapses (Charara et al., 1999; Kieval et al., 2000) (Fig. 2A-B). To determine the source of these terminals, the anterograde transport of biotinylated dextran amine (BDA) was combined with GluR6/7 or KA2 immunostaining. Following BDA injections in the centromedian thalamic nucleus or the primary motor cortex, more than half of anterogradely labelled boutons in the postcommissural putamen displayed GluR6/7 and KA2 immunoreactivity (Fig. 2C), which indicate that kainate receptors may act as pre-synaptic autoreceptors to control glutamate release from the thalamus and the cerebral cortex in the primate striatum. A particular feature of kainate receptor subunit immunoreactivity is their strong intracellular expression under basal conditions (Fig. 2B-D). Using pre- and post-embedding immunogold methods, the bulk of GluR6/7 and KA2 immunoreactivity is, indeed, associated with intracellular organelles rather than being bound to the plasma membrane (Fig. 2B-D). In immunoreactive axon terminals, kainate receptor subunit immunoreactivity is attached

to the membrane of synaptic vesicles which, in some cases, are located in the active zone of asymmetric synapses (Fig. 2D-E). Postsynaptic labelling of asymmetric postsynaptic specilaizations is also seen (Fig. 2F). Although the functions of kainate receptors in the striatum are still obscure due to the lack of specific compounds to modulate these receptors, the recent development of specific AMPA antagonists (Partenain et al., 1995) should help further our knowledge of the role of these receptors in the functional circuitry of the basal ganglia. Another promising research avenue that should definitely be explored over the next few years is the potential role of presynaptic kainate receptors in the excitotoxic phenomenon involved in the death of striatal projection neurons in Huntington's disease. The recent findings that the age of onset of Huntington's disease could, in some cases, be attributed to the genotype variation of the GluR6 kainate receptor subunit in humans strongly suggest that these receptors may, somehow, be involved in the neurodegenerative process in Huntington's patients (Rubinzstein et al., 1997; MacDonald et al., 1999).

NMDA Receptors

Irrespective of the approach used, all data agree that the human striatum is enriched in NMDA receptors. Ligand binding studies show dense NMDA binding sites throughout the whole extent of the caudate nucleus, putamen and nucleus accumbens, with a tendency to be slightly higher in the matrix than the patch compartment (Dure et al., 1992; Lee and Choi, 1992). As discussed above for the AMPA receptors, NMDA binding is affected in various brain diseases (Dure et al., 1991; Ulas et al., 1994; Noga et al., 1997; Blue et al., 1999). Data obtained in postmortem parkinsonian brains are controversial; both increases (Ulas et al., 1994) and decreases (Gerlach et al., 1996; Meoni et al., 1999) of NMDA binding sites have been reported in the caudate nucleus and putamen of these patients. Despite these changes in binding density, no major difference in NMDAR1 mRNA expression is found in the striatum of parkinsonian patients (Meoni et al., 1999). On the other hand, significant reduction in NMDA binding was

found in the striatum of Huntington's patients (Dure et al., 1991) whereas an increased density of NMDA receptors was reported in the putamen of schizophrenics (Aparicio-Legarza et al., 1998). Recent *in situ* hybridization data provide clear evidence for a differential distribution of various NMDA receptor subunits among the two populations of striatal projection neurons and interneurons (Kosinki et al., 1998; Küppenbender et al., 2000). In brief, those data indicate: (1) intense NMDAR1 and NMDAR2B signal over all striatal neurons, (2) strong NMDAR2A signal over GAD67-immunoreactive neurons, intermediate labeling over substance P-containing projection neurons, low labeling over enkephalin-positive projection neurons but no signal over somatostatinergic and cholinergic interneurons which, on the other hand, express moderate signals for NMDAR2D (3) weak NMDAR2C signal over all striatal neurons except for the moderate labeling of cholinergic interneurons and (4) low NMDAR2D labeling over GAD67- and substance P-containing neurons and no labeling over enkephalin-positive projection neurons. Furthermore, only 25% of intrastriatal dopaminergic neurons express NMDAR1 immunoreactivity in MPTP-treated monkeys (Betarbet and Greenamyre, 1999). These data highlight the fact that, although all striatal neurons express NMDA receptors, their subunit composition may significantly differ among the various neuronal populations. This provides a basis for therapeutic development aimed a targeting glutamatergic synapses associated with specific NMDA receptor subtypes in neurodegenerative diseases (see below).

Metabotropic Glutamate Receptors

As mentioned above, three main groups of mGluRs have been cloned. Antibodies have now been generated against most of these receptor subtypes which allow to study their neuronal and subsynaptic localization at the electron microscopic level. To our knowledge, data on the localization of mGluRs in the human striatum are limited to a few binding studies (Dure et al., 1991; Blue et al., 1999) and a recent immunocytochemical analysis of the distribution of mGluR2 (Phillips et al., 2000). The neuropil in both the dorsal and ventral striatum displays

strong mGluR2 immunoreactivity, but no cells or recognizable neuronal processes could be seen. This supports recent data showing that mGluR2/3 immunoreactivity in the rat striatum is mostly associated with cortical axon terminals (Testa et al., 1998).

We used polyclonal antisera raised against mGluR1a and mGluR5 (a,b) to study the subsynaptic distribution of group I mGluRs in the dorsal striatum of monkeys (Fig. 3) (Smith et al., 2000a). Overall, the pattern of group I mGluRs immunoreactivity is the same in the caudate nucleus and the putamen. Both medium-sized projection neurons and large interneurons display mGluR1a and mGluR5 immunoreactivities. In general, the neuropil staining is much more intense with the mGluR5 than the mGluR1a antiserum. No obvious patch/matrix pattern of distribution of immunoreactive neurons is observed with both antisera (Smith et al., 2000a). At the electron microscope level, the immunoperoxidase reaction product is mostly found in post-synaptic elements including large- and small-sized perikarya with smooth or indented nuclei, dendritic processes of various sizes and dendritic spines. In addition, some axon terminals that form asymmetric axo-spinous synapses display light mGluR1a immunoreactivity, but presynaptic labelling was never encountered in mGluR5-immunostained sections (Smith et al., 2000a). In sections labeled with immunogold, both mGluR1a and mGluR5 immunoreactivities are commonly found at the edges of asymmetric post-synaptic densities of axo-spinous and axo-dendritic synapses (Fig.3B,C). In the mGluR5-immunostained sections, aggregates of gold particles are also associated with the main body of symmetric post-synaptic specializations established by terminals that morphologically resemble intrinsic GABAergic boutons (Fig. 3C,D). In sections double labelled for tyrosine hydroxylase (TH) and group I mGluRs, mGluR5 immunoreactivity is occasionally found perisynaptically to symmetric synapses established by TH-containing terminals (Fig. 3A,C). A large number of gold particles are also found extrasynaptic along the membrane of dendrites and spines.

So far, very little is known about the subcellular and subsynaptic localization of group II and group III mGluRs in the primate striatum. Preliminary evidence indicates that group II mGluRs are expressed presynaptically in putative glutamatergic axon terminals and

postsynaptically in dendrites and spines (Paquet and Smith, 1997). On the other hand, group III mGluRs (mGluR7a and mGluR4a) are found in both GABAergic and glutamatergic boutons (Paquet and Smith, 1997). On the basis of these anatomical data, it appears that both pre and postsynaptic mGluRs may act at various sites to modulate glutamatergic, dopaminergic and GABAergic synaptic transmission in the primate striatum (Fig. 3). The expression of group II and group III mGluRs in glutamatergic terminals raises the interesting possibility of targeting these receptors to decrease the release of glutamate in Huntington's disease, thereby, protecting striatal projection neurons from excitotoxic cell death.

Ionotropic GABA-A Receptors

It is well established that there are two BZ binding sites on GABA-A receptors which can be differentiated by their affinity for specific agonists and antagonists (Johnson, 1996; Mehta and Ticku, 1999). The use of selective radioactive ligands for BZ1 and BZII receptors allowed to study the distribution of BZ/GABA-A receptors in the human and monkey striatum in both normal and pathological conditions. However, many studies were carried out using ligands that bind to both BZ receptor subtypes. In the description below, we will refer to either BZI or BZII receptors in cases where specific ligands were used and BZ binding sites in cases where data have been obtained with nonspecific ligands that recognize both receptor subtypes. In brief, BZ binding studies led to the following data: (1) Both receptor subtypes are significantly more abundant in the ventral than dorsal striatum (Young and Kuhar, 1979; Penney and Young, 1982; Walker et al., 1984; Faull and Villiger, 1986, 1988; Waldvogel et al. 1998, 1999), (2) In both the caudate nucleus and putamen, BZI and BZII binding sites are distributed according to the patch/matrix compartmentation in humans and monkeys (Faull and Villiger, 1986, 1988; Waldvogel et al., 1998, 1999), (3) The density of BZ binding sites is significantly decreased in the striatum of Hutington's patients (Penney and Young, 1982; Walker et al., 1984; Glass et al., 2000), (4) The density of BZ binding sites is decreased in the rostral part of the caudate nucleus

and putamen of MPTP-treated monkeys. This decrease remains unchanged after treatment with D1 or D2 receptor agonists (Calon et al., 1999) and (5) Continuous, but not pulsatile, administration of the D2 agonist, U91356A, in MPTP monkeys leads to a significant decrease of BZ binding sites in the striatum (Calon et al., 1995). PET imaging is another approach that has been used to study the *in vivo* distribution of BZ binding sites in monkey and human striatum (Brouillet et al., 1990; Moerlein and Perlmutter, 1992; Schmid et al., 1995). Results obtained so far with these methods are largely consistent with in vitro binding data.

The development of antibodies and cDNA probes raised against various GABA-A receptor subunits allowed studies of the detailed cellular localization of these subunits in the human and monkey striatum. Overall, the immunohistochemical labeling for many GABA-A receptor subunits shows a marked heterogeneous distribution, which corresponds to the patch/matrix striatal compartments, throughout the human striatum. In brief, apart from the $\alpha 1$ subunits which are more abundant in the matrix than the patch compartment, all other subunits examined ($\alpha 2$, $\alpha 3$, $\beta 2/3$, $\gamma 2$) are expressed in both compartments but significantly more in patches than the matrix in the dorsal striatum (Waldvogel et al., 1999). The $\alpha 1$ and $\beta 2/3$ subunits display a similar pattern of distribution in the baboon striatum (Waldvogel et al., 1998). It is noteworthy that the pattern of compartmental expression of these receptor subunits, except for the $\alpha 1$ subunit, is different in the ventral striatum where all subunits are preferentially expressed in the matrix compartment (Waldvogel et al., 1999). Co-localization studies using various markers of striatal neurons led to the following conclusions about GABA-A receptor subunit configurations in human striatal cells: (1) The parvalbumin/GABA interneurons are enriched in $\alpha 1$, $\beta 2/3$ and $\gamma 2$ subunits whereas the calretinin interneurons express the same subunits but also contain high levels of the $\alpha 3$ subunit, (2) The cholinergic interneurons only express the $\alpha 3$ subunit, (3) The NPY-immunoreactive neurons are completely devoid of GABA-A receptor subunit immunoreactivity and (4) The calbindin-containing projection neurons express a low to moderate level of $\alpha 2$, $\alpha 3$, $\beta 2/3$ and $\gamma 2$ subunits immunoreactivity but are devoid of $\alpha 1$ subunit labeling. In monkeys, the $\alpha 1$ subunit is also expressed preferentially in striatal interneurons

whereas the β2/3 subunits immunoreactivity is much more homogeneously distributed (Waldvogel et al., 1998). Other GABA-A receptor subunits found in the monkey striatum include the α4 and δ subunits which are expressed at high and moderate levels, respectively, in most striatal neurons (Kultas-Ilinsky et al., 1998). In contrast, the β1 and γ1 subunit mRNAs are not detectable in the monkey striatum (Kulktas-Ilinsky et al., 1998). These data indicate that the subunit composition of GABA-A receptors displays a considerable degree of regional and cellular heterogeneity in the human and monkey striatum.

So far, the electron microscopic analysis of GABA-A receptor subunits in the primate striatum is limited to immunoperoxidase localization of α1 and β2/3 subunit labeling in baboon and macaque monkeys. In both species, the GABA-A receptor subunits are expressed along the plasma membrane of striatal projection neurons and interneurons. At the subsynaptic level, peroxidase labeling was found to be associated with both symmetric and asymmetric membrane specializations as well as with nonsynaptic sites along the plasma membrane (Waldvogel et al., 1998). Further immunogold studies are essential to characterize the exact synaptic localization of GABA-A receptor subunits in the primate striatum. Recent immunogold data indicate that the α1, β2/3 and γ2 subunit immunoreactivities are expressed postsynaptically in the main bodies of symmetric GABAergic synapses in the rat striatum (Fujiyama et al., 2000).

Metabotropic GABA-B Receptors

The distribution of GABA-B binding sites in the monkey basal ganglia has recently been studied using high affinity radioactive ligands (^{3}HCGP 62349; ^{125}I-CGP 64213) and ^{3}H-GABA. The striatum and the substantia nigra were found to display the highest level of GABA-B binding in the basal ganglia (Ambardekar et al., 1999; Bowery et al., 1999; Calon et al., 2000). In general, the distribution of striatal binding sites is relatively homogeneous throughout the caudate nucleus, putamen and nucleus accumbens. No obvious patch/matrix compartmention of

labelling was noticed. The density of striatal GABA-B binding sites is not changed in MPTP-induced parkinsonian monkeys treated with dopaminergic agonists, despite a significant decrease in the substantia nigra and increased binding in the GPi (Calon et al., 2000).

The recent cloning of two GABA-B receptor subtypes (GABA-BR1 and GABA-BR2) and the subsequent development of antibodies and mRNA probes led to a better characterization of the cellular and subcellular localization of GABA-B receptors in rat (Fritschy et al., 1998; Margeta-Mitrovic et al., 1999), monkey (Charara et al., 2000a,b) and human (Makoff, 1999; Billinton et al., 2000) brain. We recently used affinity-purified polyclonal antisera to localize immunocytochemically GABA-BR1 and GABA-BR2 receptor subtypes at light and electron microscope level in the monkey basal ganglia (Charara et al., 2000a,b). Overall, the pattern of labelling generated by both antisera is relatively similar throughout the primate basal ganglia, except that the intensity of labelling is generally much higher for GABA-BR1 than GABA-BR2 immunoreactivity. The similarity in distribution for both GABA-B receptor subtypes is consistent with the idea that GABA-BR1 and GABA-BR2 receptors must form heterodimers to be functional (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999; Makoff, 1999).

In the striatum, the staining for both GABA-B receptor subtypes is homogeneous and relatively similar throughout the caudate nucleus, putamen and nucleus accumbens. Both medium-sized projection neurons and large interneurons are immunostained. In general, interneurons are more strongly labeled than projection neurons which is in keeping with recent findings showing that the GABA-BR1 immunoreactivity is particularly abundant in a small population of neurons scattered throughout the rat striatum (Margeta-Mitrovic et al., 1999). There is no patchy distribution of immunostaining, indicating that GABA-B receptor immunoreactivity is not differentially expressed in the patch-matrix compartments, which differs from the patchy distribution of some GABA-A receptor subunits in the monkey and human striatum (see above).

At the electron microscope level, the immunoperoxidase product of GABA-BR1 and GABA-BR2 is found in postsynaptic elements including large- and medium-sized cell bodies, dendrites and spines (Charara et al., 2000a,b). Occasionally, GABA-BR1 immunoreactivity is also detected in cell bodies and thin processes of astrocytes. In sections stained with the pre-embedding immunogold method, GABA-BR1 immunoreactivity was found to be localized in the main body of symmetric post-synaptic specializations established by terminal boutons packed with large pleomorphic vesicles or vesicle-filed axonal processes (Fig. 4D-E). Perisynaptic labeling at asymmetric axo-spinous and axo-dendritic synapses is also seen (Fig. 4C,E). Finally, a large number of extrasynaptic gold particles were found in neuronal perikarya, dendrites and spines (Fig. 4E). In addition to post-synaptic elements, GABA-BR1 and GABA-BR2 immunoreactivities were found in many myelinated and unmyelinated axonal segments as well as in cortical- or thalamic-like terminal boutons forming asymmetric axo-spinous and axo-dendritic synapses (Fig. 4A-B,E). In those labelled terminals, the gold particles are found in the presynaptic grid of asymmetric axospinous and axodendritic synapses (Fig. 4B). Another much rarer type of GABA-BR1-immunoreactive terminals form "en passant" symmetric synapses with immunolabeled dendrites and display the ultrastructural features of either dopaminergic terminals from the substantia nigra or GABA-containing intrinsic striatal boutons (Charara et al., 2000a) (Fig. 4E). Although functions of GABA-B receptors in the primate brain are poorly known, these anatomical data indicate that these receptors are localized to subserve both pre- and postsynaptic control of GABAergic, glutamatergic and, possibly, dopaminergic neurotransmission in the monkey striatum. Functional data in various non-primate species, indeed, suggest that activation of GABA-B receptors modulate glutamatergic and dopaminergic transmission, but the exact localization of receptors which mediate these effects remains controversial (Sawynok and LaBella, 1981; Reinmann, 1983; Wilson and Wilson, 1985; Seabrook et al., 1990; Calabresi et al., 1990, 1991; Arias-Montano et al., 1992; Nisenbaum et al., 1992, 1993; Smolders et al., 1995).

GLUTAMATE AND GABA RECEPTORS IN THE GLOBUS PALLIDUS

Ionotropic Glutamate Receptors

Both GPe and GPi show a relatively strong binding for various ionotropic glutamate receptor subtypes and are enriched in NMDA, AMPA and kainate receptor subunits in monkeys and humans (Lee and Choi, 1992; Bernard et al., 1996; Tomiyama et al., 1997; Blue et al., 1999). The four AMPA receptor subunits (GluR1-4) are expressed at a moderate to high level in GPe and GPi of rhesus monkeys and humans (Bernard et al., 1996; Paquet and Smith, 1996; Meng et al., 1997; Tomiyama et al., 1997; Ciliax et al., 1997; Betarbet et al., 2000) whereas only low levels of KA1 and KA2 subunits are detectable. On the other hand, the human GPi is devoid of GluR5-7 kainate receptors subunit mRNAs (Bernard et al., 1996). An interesting feature about AMPA receptors in the primate pallidum is the relative lack of GluR1 immunoreactivity in GPi neurons in the squirrel monkey (Paquet and Smith, 1996). This differs from data obtained in rhesus monkeys and humans, using the same antibodies, where all AMPA receptor subunits are strongly expressed in both pallidal segments (Bernard et al., 1996; Ciliax et al., 1997). The functional significance of this potential species difference between old world and new world primates regarding AMPA receptor subunit localization remains to be established.

Neurons in both pallidal segments are enriched in NMDAR1 and NMDAR2D subunit, but also display low level of expression of NMDAR2A,B,C in humans (Kosinki et al., 1998). In GPi, a subpopulation of neurons exhibiting low NMDAR2D mRNA signal can be easily separated from the majority of pallidal neurons which display intense labelling for this subunit (Kosinki et al., 1998). Taking into account that the bulk of glutamatergic afferents to the globus pallidus arises from the subthalamic nucleus, it is likely that both AMPA and NMDA receptors with different subunit composition might be expressed at subthalamopallidal synapses in primates.

Based on the functional model of basal ganglia circuitry suggesting that the STN is hyperactive in Parkinson's disease, one could hypothesize that this hyperactivity results in a decreased glutamate receptor expression in the pallidum. It was, indeed, recently shown that the GluR1 mRNA expression and protein levels are significantly decreased in GPi and SNr of parkinsonians (Bernard et al., 1996; Betarbet et al., 2000).

Metabotropic Glutamate Receptors

Although many studies have addressed the issue of mGluRs localization in the rodent pallidum, data in primates are much rarer and restricted to group I and group II mGluRs. In a recent light microscopic immunoperoxidase study, Philips et al (2000) have demonstrated that the neuropil of GPe and GPi displays a moderate to high mGluR2 immunoreactivity in humans with a slightly higher staining intensity in GPe than in GPi. In both pallidal segments, the immunoreactivity appeared to be associated with afferent fibers rather than pallidal neurons per se (Phillips et al., 2000), which suggests the existence of presynaptic group II mGluRs in the human pallidum. These data are strikingly different from those obtained in rodents which revealed low level of mGluR2 mRNAs and immunoreactivity in the rat and mouse globus pallidus (Ohishi et al., 1993; Testa et al., 1994; 1998). Whether this indicates a true species difference between rodents and primates regarding the presynaptic localization of mGluR2 in the pallidal complex or relies upon technical differences in the sensitivity of the different antibodies used in these studies remains to be established. It is worth noting that functional group II mGluRs were found to be expressed on putative STN glutamatergic terminals in the rat SNr (Bradley et al., 2000). If the intense mGluR2 immunolabeling seen in the human pallidum corresponds to presynaptic STN terminals, the group II mGluRs become a very promising target to reduce the release of glutamate from hyperactive subthalamopallidal terminals in Parkinson's disease. To further characterize this issue, electron microscopic studies are essential to determine the exact source of presynaptic mGluR2 labeling in the primate pallidum.

We recently carried out a detailed electron microscopic study of the subsynaptic localization of group I mGluRs (mGluR1a and mGluR5) in GPe and GPi of monkeys (Hanson and Smith, 1999; Smith et al., 2000a). These data indicate that both receptor subtypes are largely expressed postsynaptically in neuronal elements including dendrites and perikarya. At the subsynaptic level, mGluR1a and mGluR5 immunoreactivities are found at the edges of asymmetric postsynaptic specializations of putative glutamatergic synapses (Fig. 5B-C); a pattern of labelling consistent with that found in the rat cerebellum and hippocampus (Nusser et al., 1994; Lujan et al., 1996; Ottersen and Landsend, 1997). However, a surprising observation was a strong mGluR1a and mGluR5 labelling associated with the core of symmetric synapses established by putative GABAergic striatal terminals (Hanson and Smith, 1999) (Fig. 5A,C). A large proportion of group I mGluR labelling was also found at extrasynaptic sites along the dendrites of GPe and GPi neurons. These observations raise questions about the potential sources of glutamate that activates these receptors and their functional significance at GABAergic synapses (see below).

To our knowledge, the distribution of group III mGluRs has not yet been studied in the primate pallidum. However, data in rodents indicate that mGluR4a and mGluR7a,b are expressed pre-synaptically in striatopallidal terminals where they may modulate GABA release from the striatum (Kinoshita et al., 1998; Bradley et al., 1999; Kosinki et al., 1999). The fact that mGluR4a is selectively expressed on striatopallidal, but not striatonigral, terminals makes it an ideal target to reduce GABA release from the overactive striatopallidal projection in Parkinson's disease.

GABA-A Receptors

Overall, the density of BZ binding sites in the primate globus pallidus is much lower than in the striatum and restricted to BZI receptor subtypes (Faull and Villiger, 1988; Waldvogel et al., 1998; 1999). The level of binding is relatively higher in the ventral pallidum than the dorsal

pallidum, and more prominent in GPe than in GPi (Faull and Villiger, 1988; Waldvogel et al., 1998; 1999). Differential changes in binding intensity between the two pallidal segments were found in postmortem brains of Huntington's patients. Whereas the GPe shows an increased binding at the very early stage of the disease, the GPi is much more strongly labelled than GPe in advanced grades of Huntington's disease (Glass et al., 2000). These data are consistent with previous findings showing that striato-GPe neurons degenerate before striato-GPi neurons in Huntington's patients (see Vonsatell and DiFiglia, 1998 for a review). The increased GABA-A binding sites might reflect a compensatory mechanism for the decrease in GABA release due to striatal cell death. Changes in pallidal BZ binding sites were also noticed in animal models of Parkinson's disease. MPTP-treated monkeys show an increased level of GABA/BZ binding in the GPi which could be reversed by treatment with the long acting D2 receptor agonist, cabergoline, but not by the D1 receptor agonist, SKF 82958 (Robertson et al., 1990; Calon et al., 1995; 1999). In contrast, the density of GABA/BZ binding sites is decreased in GPe after MPTP treatment (Griffiths et al., 1990; Robertson et al., 1990). This up- and downregulation of GABA-A receptors in GPi and GPe, respectively, is consistent with the current functional model of basal ganglia circuitry which suggests that the activity of the "so-called" direct striato-GPi pathway is decreased in Parkinson's disease whereas the activity of the "indirect" striato-GPe projection is increased (Albin et al., 1989; DeLong, 1990).

As expected, based on their strong GABAergic innervation from the striatum (Shink and Smith, 1995), pallidal neurons are enriched in various GABA-A receptor subunits in primates and non-primates (Zhang et al., 1981; Fritschy and Mohler, 1995; Charara and Smith, 1998; Kultas-Ilinsky et al., 1998; Waldvogel et al., 1998, 1999). Using double labeling immunofluorescence techniques, Waldvogel et al (1999) studied the expression of various GABA-A receptor subunits in chemically characterized neurons in the human pallidum. Their main findings are: (1) Pallidal neurons are devoid of $\alpha 2$ subunit immunoreactivity, (2) GABAergic pallidal neurons immunoreactive for parvalbumin and a subpopulation of strongly immunoreactive calretinin (CR)-containing neurons express high levels of $\alpha 1$, $\alpha 3$, $\beta 2/3$ and $\gamma 2$

immunoreactivity and (3) a subpopulation of pallidal neurons which display very intense CR immunoreactivity express α1, β2/3 and γ2 subunit immunoreactivities. Findings in monkeys are consistent with those data except that the α2 mRNA is expressed at a moderate level in GPe and GPi neurons in non-human primates (Kultas-Ilinsky et al., 1998). The lack of α2 subunit in pallidal neurons seems to be a feature unique to the human pallidum since GP neurons also display α2 immunoreactivity in rats (Fritschy and Mohler, 1995). It is worth noting that monkey pallidal neurons also express moderate to high level of α4 and δ subunit mRNAs, but are devoid of γ1 subunit (Kultas-Ilinsky et al., 1998). The β1 subunit mRNA is expressed at a low level in GPe but is not detectable in the monkey GPi (Kultas-Ilinsky et al., 1998). The subcellular localization of α1 and β2/3 subunit immunoreactivity has been studied at the electron microscope level using pre-embedding immunoperoxidase methods (Charara and Smith, 1998; Waldvogel et al., 1998). Both antibodies resulted in intense labeling of the plasma membrane of GPe and GPi neurons. In some cases, aggregates of peroxidase reaction product were associated with symmetric and asymmetric post-synaptic specializations (Waldvogel et al., 1998), suggesting the existence of GABA-A receptor subunits at both GABAergic and putative glutamatergic synapses. Strong labeling was also found at non-synaptic sites along the plasma membrane. To further characterize the subsynaptic localization of the GABA-A receptor subunits in the monkey pallidum, we started a series of electron microscopic post-embedding immunogold studies of α1, β2/3 and γ2 subunits immunoreactivity in rhesus monkeys. So far, our data indicate that the α1 and β2/3 subunit immunoreactivity is confined to the main body of symmetric synapses (Figs. 6A,7), which is consistent with recent data in rodents using the same approach (Fujiyama et al., 1998).

GABA-B Receptors

Low to moderate levels of GABA-B binding sites are expressed in both pallidal segments in normal monkeys (Ambardekar et al., 1999; Bowery et al., 1999). After MPTP treatment, the

level of GABA-B receptors is significantly increased in GPi, but no changes are seen in GPe, striatum and SNr (Calon et al., 2000). The increase in GPi is not affected by treatment with D1 dopamine receptor agonist, but is partly reversed by cabergoline, a potent D2 dopamine receptor agonist (Calon et al., 2000).

Recent immunohistochemical studies revealed the existence of both GABA-BR1 and GABA-BR2 receptors immunoreactivity in virtually all pallidal neurons in humans (Billinton et al., 2000) and monkeys (Charara et al., 2000a,b). Overall, the pattern of both GABA-B receptor subtypes is the same throughout GPe and GPi except that the intensity of labelling for GABA-BR1 is much stronger than that of GABA-BR2.

At the electron microscope level, GABA-BR1 and GABA-BR2 immunoreactivity is enriched in post-synaptic neuronal elements including perikarya and dendritic shafts of various sizes (Charara et al., 200aa,b). At the subsynaptic level, GABA-BR1 immunoreactivity is commonly found in the main body of symmetric synapses established by striatal-like GABAergic terminals in both GPe and GPi or at the edges of asymmetric post-synaptic specializations of axo-dendritic synapses (Figs. 6 B-C, 7). Extrasynaptic labeling is also detected in neuronal perikarya and dendrites. The most striking feature of GABA-BR1 and GABA-BR2 immunoreactivity in the GPe and GPi is the pre-synaptic labeling of numerous unmyelinated axonal segments and putative STN-like glutamatergic terminals forming asymmetric synapses (Charara et al., 2000a,b) (Figs. 6D, 7), suggesting that GABA-B act as heteroreceptors to modulate glutamate release in the globus pallidus. Another population of lightly labeled terminals that display the ultrastructural features of striatal boutons and form symmetric axo-dendritic synapses also display GABA-BR1 and GABA-BR2 immunoreactivity in both pallidal segments.

GLUTAMATE AND GABA RECEPTORS IN THE SUBTHALAMIC NUCLEUS

Ionotropic and Metabotropic Glutamate Receptors

Very little is known about ionotropic glutamate receptor localization in the primate STN. Binding studies indicate that NMDA, AMPA and kainate receptors are expressed at a low to moderate level in this brain region in humans (Lee and Choi, 1992; Ball et al., 1994). Immunocytochemical studies revealed the existence of strong neuronal labeling for the AMPA GluR1 subunit in monkeys (Ciliax et al., 1997). Nigrostriatal dopaminergic denervation does not induce significant changes in GluR1 protein expression in STN neurons of MPTP-treated monkeys (Betarbet et al., 2000). Although immunohistochemical studies of other ionotropic glutamate receptor subunits have not been carried out in the primate STN, data in rodents indicate that various types of NMDA, AMPA and kainate receptor subunits are expressed in this brain structure (Petralia and Wenthold, 1992; Petralia et al., 1994a,b; Standaert et al., 1994; Bishoff et al., 1997). At the subsynaptic level, AMPA and NMDA receptor subunits are co-expressed in the core of asymmetric synapses, though some of the AMPA GluR2/3 immunoreactivity is also associated with non-synaptic sites and symmetric synaptic junctions in rats (Clarke and Bolam, 1998). Such information has not yet been gathered in primates.

Group I and group II mGluRs are expressed in the primate STN. Immunoreactivity for mGluR2 is far less intense in the STN than other parts of the basal ganglia in humans (Phillips et al., 2000). This is surprising since STN neurons show high level of mGluR2 mRNA expression in the rat (Testa et al., 1994). Although neuronal perikarya are lightly labeled, the neuropil of the STN displays strong immunoreactivity for both mGluR1a and mGluR5 in monkeys. At the electron microscope level, both group I mGluRs are found almost exclusively in post-synaptic elements. Immunogold particles are commonly found at the edges of symmetric and asymmetric post-synaptic specializations (Fig. 8A,C). Interestingly, labelling is also found at the edges of puncta adherentia between putative GABAergic GPe terminals and STN dendrites (Fig. 8D). In

some cases, gold particles are also associated with the main body of "en passant type" symmetric synapses established by vesicle-filled axon-like processes or at adherent junctions between putative glial processes and neuronal structures (Fig. 8B,D). Extrasynaptic labeling is frequently found for both receptor subtypes. The group III mGluRs localization has not been studied in primates, but in situ hybridization and immunocytochemical data indicate that the mGluR4 and mGluR7 expression is very low in the rat STN (Ohishi et al., 1995; Bradley et al., 1999; Kosinki et al., 1999)

Ionotropic and Metabotropic GABA Receptors

In the monkey, STN neurons express high level of mRNA for the α1, α3, β2, β3, γ2 and δ subunits of the GABA-A receptors, but are devoid of α2, α4, β1 and γ1 subunits (Kultas-Ilinsky et al., 1998). A recent study showed that the human STN is particularly enriched in the ε subunit (Davies et al., 1997). Although STN neurons also display strong immunoreactivity for various GABA-A receptor subunits in rats and monkeys (Zhang et al., 1991; Wisden et al., 1992; Fritschy and Möhler, 1995; Charara and Smith, 1998), there is some discrepancy between rodents and primates regarding the type of GABA-A receptor subunits expressed in this nucleus. For instance, the α2 subunit immunoreactivity is abundant in rat STN neurons whereas the mRNA encoding this subunit is not detectable in the monkey STN. In contrast, the δ subunit mRNA is abundant in the monkey STN but the immunoreactivity for this protein does not reach a detectable level in rats (Fritschy and Möhler, 1995). Whether these data indicate a real species difference in the subunit composition of GABA-A receptors between rodents and primates remains to be established.

STN neurons display low to moderate immunoreactivity for GABA-BR1 and GABA-BR2 subtypes in monkey and human STN (Billinton et al., 2000; Charara et al., 2000a,b). At the electron microscope level, both receptor subtypes are expressed post-synaptically on dendrites of STN neurons and pre-synaptically in putative glutamatergic axon terminals in monkeys (Charara

et al. 2000a,b) (Fig. 8E). As was found in other basal ganglia structures, the GABA-BR2 immunoreactivity is far less intense than the GABAR1 immunostaining in the STN (Charara et al., 2000a,b). Together, these data indicate that both GABA-A and GABA-B receptors are likely to mediate postsynaptic inhibition by GPe in STN neurons. In addition, GABA-B receptors may also control the activity of STN neurons by presynaptic inhibition of neurotransmitter release from extrinsic and/or intrinsic glutamatergic terminals.

GLUTAMATE AND GABA RECEPTORS IN THE SUBSTANTIA NIGRA

Ionotropic and Metabotropic Glutamate Receptors in Midbrain Dopaminergic Neurons

Glutamate plays a major role in controlling the firing rate and firing pattern of midbrain dopaminergic neurons in rats (Grace and Bunney, 1984; Smith and Grace, 1992). On the other hand, glutamate may also become excitotoxic to dopaminergic neurons in Parkinson's disease. Although the exact mechanisms underlying this excitotoxic phenomenon still remains to be established, there is increasing evidence that an intracellular rise in calcium via NMDA receptor activation might be involved (Blandini et al., 1996). If such is the case, one would expect neurons in the ventral tegmental area (VTA) and dorsal tier of the SNc, which are less sensitive to neurodegeneration in PD, to express a lower level of Ca^{+2}-permeable NMDA receptors than the highly sensitive ventral tier SNc neurons. Recent *in situ* hybridization data in humans indicate that such is not the case (Counihan et al., 1998). The levels of NMDAR1 and NMDAR2 (a-d) subunit mRNAs are not significantly different between the various midbrain dopaminergic cell groups, except that neurons of the pars lateralis express a slightly higher level of labelling for all NMDA receptor subunits examined (Counihan et al., 1998). These data also show that the NMDAR2D is, by far, the most abundant NMDAR2 subunit expressed in the different subgroups of SNc neurons. On the other hand, data in squirrel monkeys demonstrate that the NMDAR1 subunit mRNA expression is significantly higher in ventral tier SNc neurons than in the dorsal

tier of the SNc and VTA (Paquet et al., 1997). Similarly, the AMPA GluR2 subunit is more abundant in ventral than dorsal SNc neurons whereas the GluR1 subunit is homogeneously distributed among midbrain dopaminergic cell groups (Paquet et al., 1997). These mRNA data are consistent with immunohistochemical findings showing that SNc and VTA dopaminergic neurons display moderate to strong immunoreactivity for the NMDAR1 and the AMPA (GluR1, GluR2/3 and GluR4) glutamate receptor subunits in monkeys (Paquet et al., 1997). However, SNc/VTA neurons are almost completely devoid of NMDAR2 A/B immunoreactivity, which is in line with recent rodent data (Standaert et al., 1994; Albers et al., 1999). At the subcellular level, the GluR1, GluR2/3 and NMDAR1 immunoreactivity is mostly associated with postsynaptic elements, though a small number of preterminal axons, axon terminals and glial cell processes are also labelled (Paquet et al., 1997).

Both group I mGluR subtypes (mGluR1a and mGluR5) are expressed in midbrain dopaminergic neurons in monkeys. Analysis of immunogold labelling at the electron microscopic level revealed that both receptor subtypes are mostly expressed postsynaptically: (1) at the edges of asymmetric post-synaptic specializations (Fig. 9A), (2) in the main body of symmetric, putative GABAergic, synapses (Fig. 9B) and (3) extrasynaptically along the neuronal plasma membrane. A major difference between the subcellular distribution of mGluR5 and mGluR1a immunoreactivity is that a large part of mGluR1a labelling is bound to the plasma membrane whereas most mGluR5 immunostaining is intracellular. This differential distribution seems to be a common feature for the two group I mGluR subtypes in both SNr and SNc in rats and monkeys (Hubert et al., 1999). The functional significance of this large internalized pool of mGluR5 under basal conditions remains to be established. The dopaminergic neurons do not show any detectable mGluR2 immunoreactivity in the human substantia nigra (Phillips et al., 2000). Although group III mGluR localization has not been studied in the primate SN, recent data indicate that both SNc and SNr neurons do not express detectable levels of mGluR4 and mGluR7 immunoreactivity or mRNAs in rats (Ohishi et al., 1995; Bradley et al., 1999; Kosinki et al., 1999).

GABA-A and GABA-B Receptors in Midbrain Dopaminergic Neurons

Midbrain dopaminergic neurons in the SNc express varying degrees of GABA-A receptor subunits in monkeys. The α1 and α2 subunits are expressed at a low level whereas the α3, α4, β1, β2, β3, γ2 and δ are much more abundant. It is worth noting that only SNc neurons express the β1 subunit mRNA in monkey basal ganglia (Kultas-Ilinsky et al., 1998). As is the case for most basal ganglia structures, SNc neurons do not express a detectable level of γ1 subunit mRNA.

The SNc displays the highest GABA-B receptor binding site densities in the monkey basal ganglia (Ambardekar et al., 1999) and expresses strong and moderate GABA-B R1 and GABA-B R2 immunoreactivity, respectively (Billinton et al., 2000; Charara et al., 2000a,b). MPTP lesion of dopaminergic neurons results in a significant loss of GABA-B binding sites in the monkey SNc, suggesting that GABA-B receptors are largely expressed on SNc neurons (Calon et al., 2000). At the electron microscopic level, immunoreactivity for both GABA-B receptor subtypes is, indeed, largely found postsynaptically in neuronal perikarya and dendrites. Rare pre-terminal axons and terminal boutons forming asymmetric synapses are occasionally labelled (Charara et al., 2000a,b) (Fig. 9C).

Ionotropic and Metabotropic Glutamate Receptors in SNr GABAergic Neurons

The pattern of distribution of ionotropic glutamate receptors in SNr neurons is similar to that seen in the globus pallidus. Furthermore, as is found in the monkey GPi, the expression of the AMPA receptor subunit, GluR1, is downregulated in SNr neurons of parkinsonian monkeys (Betarbet et al., 2000).

GABA-A and GABA-B Receptors in SNr GABAergic Neurons

The pattern of GABA-A/BZ binding sites in the SNr and the expression of GABA-A receptor subunits is largely similar to that in the globus pallidus (Robertson et al.,1989) (see above), except for the expression of the α4 subunit mRNA which is abundant in GPi but not detectable in the monkey SNr (Kultas-Ilinsky et al., 1998). Following MPTP treatment, a significant increase in GABA-A/BZ binding sites is observed in the monkey SNr (Robertson et al., 1989), which is consistent with the hypothesis that the "direct" striatonigral GABAergic pathway is underactive in Parkinson's disease (DeLong, 1990). Although a detailed electron microscopic analysis of the subsynaptic localization of these receptors has not yet been carried out in primates, recent immunogold data indicate that the bulk of GABA-A receptor subunits are expressed in the core of symmetric striatonigral synapses in rats (Fujiyama et al., 1998).

In general, the SNr displays a low level of GABA-B receptor binding sites (Ambardekar et al., 1999) and is lightly labelled with GABA-BR1 and GABA-BR2 receptor antibodies in monkeys (Charara et al., 2000a,b) and humans (Billinton et al., 2000). No change in GABA-B receptor binding is seen in the SNr following MPTP treatment (Calon et al., 2000). The pattern of subcellular distribution of GABA-B receptor subtypes in the monkey SNr resembles that seen in the globus pallidus, i.e. dendrites and many preterminal unmyelinated axons as well as a population of STN-like terminals forming asymmetric synapses display GABA-BR1 and GABA-BR2 immunoreactivity (Charara et al., 2000a,b) (Fig. 9D). Another population of striatal-like boutons display immunoreactivity for both receptor subtypes (Charara et al., 2000a,b).

POTENTIAL SOURCES OF ACTIVATION OF METABOTROPIC RECEPTORS

One of the main features which characterizes the subsynaptic localization of metabotropic glutamate and GABA receptors is their strong expression at non-synaptic sites. In fact, this pattern of distribution was also found for other types of G-protein-coupled receptors such as dopamine, muscarinic and opiate receptors (Yung et al., 1995; Svingos et al., 1997;

Bernard et al., 1998, Muriel et al., 1999). These data suggest that extrasynaptic spillover of neurotransmitter or neuropeptides might be a common mechanism to activate G protein-coupled receptors in the CNS.

One of the most surprising findings presented in this review was the localization of group I mGluRs at putative GABAergic striatal synapses in GPe, GPi and SNr (Hanson and Smith, 1999). This raises questions about the sources of activation and potential functions of these receptors at GABAergic synapses. A first possible source of glutamate might be the spillover of transmitter released from glutamatergic terminals. Extrasynaptic diffusion of glutamate to activate AMPA and NMDA receptors was, indeed, demonstrated in the rat hippocampus and cerebellum (Asztely et al., 1997; Barbour and Hausser, 1997; Kullmann and Asztely, 1998; Dzubay and Jahr, 1999). Since mGluRs display a stronger affinity for glutamate than ionotropic receptors (Conn and Pin, 1997), it is likely that even a small amount of spilled over neurotransmitter is enough to induce mGluRs activation. Another possibility is that glutamate released from astrocytes activates mGluRs located at symmetric synapses and, possibly, those located extrasynaptically. Data obtained over the past few years showing that astrocytes express various ion channels and contain glutamate receptors (Sontheimer et al, 1996; Steinhauser and Gallo, 1996; Verkhratsky and Kettenmann, 1996; Porter and McCarthy, 1997; Carmignoto et al., 1998), have shifted the traditional concept of astrocytes as simple structural support for neurons to a view in which glial cells play a more active role in information processing and neuronal communication in the central nervous system (Parpura et al., 1994; Antanitus, 1998). It is well established that neuronal stimulation induces waves of elevated intracellular calcium which propagate between glial cells and lead to glutamate release (Parpura et al., 1994; Araque et al., 1999). A third possibility is that striatal terminals, under certain circumstances, release excitatory amino acids. Although this is not consistent with the current view of neurotransmission at striatofugal synapses, indirect evidence suggests that striatal neurons may co-express, and possibly co-release, GABA and glutamate as neurotransmitters. First, striatopallidal neurons possess a high-affinity uptake system for glutamate and aspartate (White et al., 1994). Second,

excitatory post-synaptic currents sensitive to the glutamate antagonist CNQX are found in cultures consisting only of dissociated striatal neurons (Dubinsky, 1989). Third, in vivo stimulation of the caudate nucleus produces a combination of excitatory (EPSPs) and inhibitory (IPSPs) post-synaptic potentials in the rat globus pallidus (Levine et al., 1974; Kita and Kitai, 1991). Although these excitatory effects can be attributed to activation of axons extrinsic to the striatum or multisynaptic pathways, they might also originate from intrinsic striatal neurons. Even if co-release of fast neurotransmitters such as glutamate and GABA is clearly not a common feature in the CNS, the synaptic co-release of GABA and glycine was shown in the spinal cord (Jonas et al., 1998). Furthermore, Jo and Schlichter (1999) recently showed that the fast excitatory neurotransmitter ATP is co-released with the inhibitory neurotransmitter GABA at individual synapses in cultured spinal neurons. If glutamate, indeed, activates mGluRs at GABAergic synapses, it is likely that the post-synaptic mGluR responses regulate GABA currents in pallidal neurons either by changing membrane excitability through modulation of calcium and potassium channels (Conn and Pin, 1997) or via direct physical interactions with GABA-A or GABA-B receptors as was recently shown in vitro for dopamine D5 and GABA-A receptors (Liu et al., 2000). Modulatory effects of GABAergic transmission by mGluRs were shown in various brain regions in the rat including the spinal cord, the nucleus of the solitary tract, the substantia nigra pars compacta and the hippocampus (Glaum and Miller, 1993; 1994; Bonci et al., 1997; Morishita et al., 1998).

Another surprising observation made in our studies was the localization of pre- and postsynaptic GABA-BR1 and GABA-BR2 receptors immunoreactivity associated with putative glutamatergic terminals. That axo-axonic synapses are very rare in the basal ganglia rule out the hypothesis of direct synaptic release of GABA to activate these receptors. Another possibility would be that, once released, GABA diffuses out of the synaptic cleft and activates extra- and presynaptic GABA-B heteroreceptors (Attwell et al., 1993). Evidence for such a paracrine mode of GABA-B receptor activation was, indeed, demonstrated in the rat hippocampus (Isaacson et al., 1993). The efficacy of such a non-specific mode of transmission largely depends on the

extent to which GABA can diffuse and the affinity of GABA-B receptors for its transmitter. Although such information is still lacking for basal ganglia structures, it is worth noting that presynaptic GABA-B receptors were found to have a much higher affinity for GABA than GABA-A receptors in the rat hippocampus (Yoon and Rothman, 1991).

METABOTROPIC GLUTAMATE AND GABA-B RECEPTORS: NOVEL THERAPEUTIC TARGETS FOR PARKINSON'S DISEASE

An imbalance of activity between the direct and indirect striatofugal pathways in favor of the indirect pathway is thought to underlie most symptoms of Parkinson's disease (DeLong, 1990). The increased activity of the glutamatergic subthalamopallidal and, possibly, corticostriatal projections in animal models of Parkinson's disease led various groups to test the potential therapeutic benefits of ionotropic glutamate receptor antagonists in alleviating parkinsonian symptoms (see Starr, 1995; Blandini and Greenamyre, 1996 for reviews). Systemic administration of NMDA and non-NMDA antagonists with subthreshold doses of L-DOPA or D2 dopamine receptor agonist has proven to ameliorate symptoms in primate models of Parkinson's disease (Starr, 1995; Blandini et al., 1996). Data reported in this review strongly suggest that interactions with metabotropic glutamate and GABA-B receptors may also have beneficial effects in Parkinson's disease. Drugs interacting with these receptors are expected to influence the induction and progression of the symptoms of the disease without hampering the efficiency of fast glutamatergic and GABAergic synaptic transmission, thereby, reducing unwanted side effects commonly seen with drugs that target ionotropic receptors (Starr, 1995).

The group I mGluRs located perisynaptically at STN synapses in GPe and GPi should be considered as a potential target in PD because the perisynaptic mGluR1a and mGluR5 are likely to be activated by excessive amounts of glutamate released during hyperactivity of subthalamopallidal synapses in parkinsonians. Group I mGluR activation might, then, lead to increased activity of basal ganglia output neurons through various mechanisms including

potentiation of ionotropic glutamatergic transmission, reduction of K^+ conductances etc....(see Conn and Pin, 1997 for details). Group I mGluR antagonists should, therefore, reduce the over-excitatory drive generated by the STN in pallidal neurons.

Based on rodent data, activation of the group III mGluRs, mGluR4, seems to be an ideal strategy to alleviate symptoms of Parkinson's disease. It is well established that activation of presynaptic group III mGluRs reduces neurotransmitter release in the hippocampus (Conn and Pin, 1997). If such is also the case in GP, activation of these receptors in parkinsonians should reduce the activity of the overactive indirect pathway by reducing GABA release at striatopallidal synapses, thereby inhibiting subthalamopallidal neurons which, in turn, relieve basal ganglia output neurons in GPi and SNr from their tonic excitatory drive. The final outcome of such therapy should be an increased activity of thalamocortical neurons and facilitation of motor behaviors.

Another mGluR subtype of interest for PD therapy is mGluR2, which was found to be expressed on subthalamonigral terminals in rats (Bradley et al., 2000). Furthermore, activation of these receptors in brain slices reduces glutamatergic transmission at subthalamonigral synapses (Bradley et al., 2000) and systemic administration of group II agonist reverses haloperidol-induced catalepsy (Bradley et al., 2000).

So far, only a few specific agents for group II (LY354740 and LY379268) and group I (MPEP) mGluRs were found to produce central pharmacological actions when administered systemically in animals (Helton et al., 1998; Moghaddam and Adams, 1998; Bordi and Ugolini, 1999; Schoepp et al., 1999). However, the potential therapeutic benefit of such agents will likely drive the development of additional compounds that could be administered systemically for novel medical purposes.

The expression of GABA-B receptors in subthalamopallidal and subthalamonigral terminals (Charara et al., 2000a,b) suggests that activation of these pre-synaptic heteroreceptors might attenuate the overflow of glutamate released by STN neurons in Parkinson's disease. In support of this hypothesis, application of baclofen was found to reduce the evoked synaptic

currents mediated by glutamate in the rat SNr *in vitro* (Shen and Johnson, 1997). The current use of GABA-B agonists in therapeutics is mostly restricted to baclofen in the treatment of spasticity (Porter, 1997). In fact, the beneficial antispastic effect of baclofen is believed to derive from the suppression of excitatory neurotransmitter release to motoneurons in the spinal cord (Fox et al., 1978; Davies, 1981; Bonnano et al., 1998). Future behavioural studies of baclofen in animal models should help ascertain the potential therapeutic efficacy of this drug for Parkinson's disease and understand better the functions of GABA-B in modulating glutamatergic neurotransmission in the basal ganglia circuitry. Interestingly, baclofen was found to reduce haloperidol-induced dyskinesias without causing gross motor depression in squirrel monkeys (Neale et al., 1984).

CONCLUDING REMARKS

The data reviewed in this paper highlight the complexity of GABAergic and glutamatergic synaptic transmission in the primate basal ganglia. The rather unusual pattern of subsynaptic localization of mGluRs and GABA-B receptors in various basal ganglia structures suggests that activation of these receptors may mediate complex presynaptic heteroreceptor functions and/or induce postsynaptic receptor interactions with other neurotransmitter receptor subtypes. These observations combined with recent evidence for the extrasynaptic diffusion of GABA and glutamate in the CNS clearly indicate that the activation of metabotropic receptors is likely to be far more complex than the current concept of synaptic receptor activation largely based on ionotropic receptor studies. A better understanding of GABAergic and glutamatergic transmission in normal and pathological basal ganglia functions surely relies upon further analyses of the anatomical localization, physiological effects and pharmacological properties of mGluR and GABA-B receptor subtypes in the primate basal ganglia.

ACKNOWLEDGMENTS

The authors thank Jean-François Paré and Rachel Kotys for technical assistance, Frank Kiernan for photography and Peggy Plant for clerical work. This work was supported by NIH grants R01 NS37423-01, P50 NS38399-01 and RR00165 as well as grants for the Parkinson's Disease Foundation and the US Army.

REFERENCES

Albers, D.S., Weiss, S.W., Iadarola, M.J., Standaert, D.G. (1999) Immunohistochemical localization of N-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor subunits in the substantia nigra pars compacta of the rat. Neuroscience 89, 209-220.

Albin, R.L., Young, A.B., Penney, J.B. (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci. 12, 366-375.

Ambardekar, A.V., Ilinsky, I.A., Froestl, W., Bowery, N.G., Kultas-Ilinsky, K. (1999) Distribution and properties of $GABA_B$ antagonist [^{3}H]CGP 62349 binding in the rhesus monkey thalamus and basal ganglia and the influence of lesions in the reticular thalamic nucleus. Neuroscience 93, 1339-1347.

Antanitus, D.S. (1998) A theory of cortical neuron-astrocyte interaction. Neuroscientist 4, 154-159.

Aparicio-Legarza, M.I., Davis, B., Hutson, P.H., Reynolds, G.P. (1998) Increased density of glutamate/N-methyl-D-aspartate receptors in putamen from schizophrenic patients. Neurosci. Lett. 241, 143-146.

Araque, A., Parpura, V., Sanzgiri, R., Haydon, P.G. (1999) Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. 22, 208-215.

Arias-Montano, J.A., Martinez-Fong, D., Aceves, J. (1992) $GABA_B$ receptor activation partially inhibits N-methyl-D-aspartate-mediated tyrosine hydroxylase stimulation in rat striatal slices. Eur. J. Pharmacol. 218, 335-338.

Asztely, F., Erdemli, G., Kullmann, D.M. (1997) Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake. Neuron 18, 281-293.

Attwell, D., Barbour, B., Szatkowski, M. (1993) Nonvesicular release of nerotransmitter. Neuron 11, 401-407.

Ball, E.F., Shaw, P.J., Ince, P.G., Johnson, M. (1994) The distribution of excitatory amino acid receptors in the normal human midbrain and basal ganglia with implications for Parkinson's disease: a quantitative autoradiographic study using [^{3}H]glycine, [^{3}H]CNQX and [^{3}H]kainate. Brain Res. 658, 209-218.

Barbour, B., Hausser, M. (1997) Intersynaptic diffusion of neurotransmitter. Trends Neurosci. 20, 377-384.

Baude, A., Nusser, Z., Molnár, E., McIlhinney, R.A.J., Somogyi, P. (1995) High-resolution immunogold localization of AMPA type glutamate receptor subunits at synaptic and non-synaptic sites in rat hippocampus. Neuroscience 69, 1031-1055.

Baude, A., Nusser, Z., Roberts, J.D., Mulvihill, E., McIlhinney, R.A., Somogyi, P. (1993) The metabotropic glutamate receptor (mGluR1α) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. Neuron 11, 771-787.

Bergman, H., Wichmann, T., DeLong, M.R. (1990) Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. Science 249, 1436-1438.

Bernard, V., Gardiol, A., Faucheux, B., Bloch, B., Agid, Y., Hirsch, E.C. (1996) Expression of glutamate receptors in the human and rat basal ganglia: effect of the dopaminergic denervation on AMPA receptor gene expression in the striatopallidal complex in Parkinson's disease and rat with 6-OHDA lesion. J. Comp. Neurol. 368, 553-568.

Bernard, V., Bolam, J.P. (1998) Subcellular and subsynaptic distribution of NR1 subunit of the NMDA receptor in the neostriatum and globus pallidus of the rat: co-localization at synapses with the GluR2/3 subunit of the AMPA receptor. Eur. J. Neurosci. 10, 3721-3736.

Bernard, V., Laribi, O., Levey, A.I., Bloch, B. (1998) Subcellular redistribution of m2 muscarinic acetylcholine receptors in striatal interneurons in vivo after acute cholinergic stimulation. J. Neurosci. 18, 10207-10218.

Bernard, V., Somogyi, P., Bolam, J.P. (1997) Cellular, subcellular, and subsynaptic distribution of AMPA-type glutamate receptor subunits in the neostriatum of the rat. J. Neurosci. 17, 819-833.

Betarbet, R., Greenamyre, J.T. (1999) Differential expression of glutamate receptors by the dopaminergic neurons of the primate striatum. Exp. Neurol. 159, 401-408.

Betarbet, R., Porter, R.H., Greenamyre, J.T. (2000) GluR1 glutamate receptor subunit is regulated differentially in the primate basal ganglia following nigrostriatal dopamine denervation. J. Neurochem. 74, 1166-1174.

Bettler, B., Kaupmann, K., Bowery, N. (1998) $GABA_B$ receptors: drugs meet clones. Curr. Opin. Neurobiol. 8, 345-350.

Billinton, A., Ige, A.O., Wise, A., White, J.H., Disney, G.H., Marshall, F.H., Waldvogel, H.J., Faull, R.L.M., Emson, P.C. (2000) $GABA_B$ receptor heterodimer-component localisation in human brain. Mol. Brain Res. 77, 111-124.

Bischoff, S., Barhanin, J., Bettler, B., Mulle, C., Heinemann, S. (1997) Spatial distribution of kainate receptor subunit mRNA in the mouse basal ganglia and ventral mesencephalon. J. Comp. Neurol. 379, 541-562.

Bittiger, H., Froestl, W., Mickel, S., Olpe, H.R. (1993) $GABA_B$ receptor antagonists: from synthesis to therapeutic applications. Trends Pharmacol. Sci. 14, 391-394.

Blandini, F., Porter, H.P., Greenamyre, J.T. (1996) Glutamate and Parkinson's disease. Mol. Neurobiol. 12, 73-94.

Blue, M.E., Naidu, S., Johnston, M.V. (1999) Altered development of glutamate and GABA receptors in the basal ganglia of girls with Rett syndrome. Exp. Neurol. 156, 345-352.

Bolam, J.P., Hanley, J.J., Booth, P.A.C., Bevan, M.D. (2000) Synaptic organisation of the basal ganglia. J. Anat. 196, 527-542.

Bonanno, G., Fassio, A., Sala, R., Schmid, G., Raiteri, M., (1998) GABAB receptors as potential targets for drugs able to prevent excessive excitatory amino acid transmission in the spinal cord. Eur. J. Pharmacol. 362, 143-148.

Bonci, A., Grillner, P., Siniscalchi, A., Mercuri, N.B., Bernardi, G. (1997) Glutamate metabotropic receptor agonists depress excitatory and inhibitory transmission on rat mesencephalic principal neurons. Eur. J. Neurosci. 9, 143-148.

Bonnert, T.P., McKernan, R.M., Farrar, S., Le Bourdellès, B., Heavens, R.P., Smith, D.W., Hewson, L., Rigby, M.R., Sirinathsinfhji, D.J.S., Brown, N., Wafford, K.A., Whiting, P.J. (1999) Theta, a novel gamma-aminobutyric acid type A receptor subunit. Proc. Natl. Acad. Sci. USA 96, 9891-9896.

Bordi, F., Ugolini, A. (1999) Group I metabotropic glutamate receptors: Implications for brain diseases. Prog. Neurobiol. 59, 55-79.

Bormann, J. (1997) Electrophysiology of $GABA_A$ and $GABA_B$ receptor subtypes. Trends Neurosci. 11, 112-116.

Bowery, N.G. (1997) Pharmacology of mammalian $GABA_B$ receptors. In The GABA Receptors, Second Edition (eds. Enna, s.J. and Bowery, N.G.) pp. 209-236, Humana Press, Totowa, NJ.

Bowery, N.G., Hill, D.R., Hudson, A.L., Doble, A., Middlemiss, D.N., Shaw, J., Turnbull, M. (1980) (-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature 283, 92-94.

Bowery, N.G., Parry, K., Goodrich, G., Ilinsky, I., Kultas-Ilinsky, K. (1999) Distribution of $GABA_B$ binding sites in the thalamus and basal ganglia of the rhesus monkey (*Macaca mulatta*). Neuropharmacology 38, 1675-1682.

Bradley, S.R., Standaert ,D.G., Rhodes, K.J., Rees, H.J., Testa, C.M., Levey, A.I., Conn, P.J. (1999) Immunohistochemical localization of subtype 4a metabotropic glutamate receptors in the rat and mouse basal ganglia. J. Comp. Neurol. 407, 33-46.

Bradley, S.R., Marino, M.J., Wittmann, M., Rouse, S.T., Awad, H., Levey, A.I., Conn, P.J. (2000) Activation of group II metabotropic glutamate receptors inhibits synaptic excitation of the substantia nigra pars reticulata. J. Neurosci. 20, 3085-3094.

Brouillet, E., Chavoix, C., Khalili-Varasteh, M., Bottlaender, M., Hantraye, P., Yorke, J.C., Maziere, M. (1990) Quantitative evaluation of benzodiazepine receptors in live *Papio papio* baboons using positron emission tomography. Mol. Pharmaol. 38, 445-450.

Brown, R.G., Marsden, C.D. (1984) How common is dementia in Parkinson's disease? Lancet 2, 1262-1265.

Brown, R.G., Marsden, C.D. (1988) Subcortical dementia: the neurophysiological evidence. Neuroscience 25, 363-387.

Calabresi, P., Mercuri, N.B., Demurtas, M., Bernardi, G. (1990) Endogenous GABA mediates presynaptic inhibition of spontaneous and evoked excitatory synaptic potentials in the rat neostriatum. Neurosci. Lett 118, 99-102.

Calabresi, P., Mercuri, N.B., Demurtas, M., Bernardi, G. (1991) Involvement of GABA systems in feedback regulation of glutamate- and GABA-mediated synaptic potentials in rat neostriatum. J. Neurophysiol. 440, 581-599.

Calon, F., Goulet, M., Blanchet, P.J., Martel, J.C., Piercey, M.F., Bedard, P.J., Di Paolo, T. (1995) Levodopa or D2 agonist induced dyskinesia in MPTP monkeys: correlation with changes in dopamine and $GABA_A$ receptors in the striatopallidal complex. Brain Res. 680, 43-52.

Calon, F., Morissette, M., Goulet, M., Grondin, R., Blanchet, P.J., Bédard, P.J., Di Paolo, T. (1999) Chronic D1 and D2 dopaminomimetic treatment of MPTP-denervated monkeys: effects on basal ganglia $GABA_A$/benzodiazepine receptor complex and GABA content. Neurochem. Int. 35, 81-91.

Calon, F., Morissette, M., Goulet, M., Grondin, R., Blanchet, P.J., Bédard, P.J., Di Paolo, T. (2000) ^{125}I-CGP 64213 binding to $GABA_B$ receptors in the brain of monkeys: effect of MPTP and dopaminomimetic treatments. Exp. Neurol. 163, 191-199.

Carmignoto, G., Pasti, L., Pozzan, T. (1998) On the role of voltage-dependent calcium channels in calcium signaling of astrocytes *in situ*. J. Neurosci. 18, 4637-4645.

Charara, A., Blankstein, E., Smith, Y. (1999) Presynaptic kainate receptors in the monkey striatum. Neuroscience 91, 1195-1200.

Charara, A, Heilman, C, Levey, A.I. (2000a) Pre- and post-synaptic $GABA_B$ receptors in the basal ganglia in monkeys. Neuroscience 95, 127-140.

Charara, A., Smith, Y. (1998) Subsynaptic distribution of $GABA_A$ receptor subunits in the globus pallidus and subthalamic nucleus in monkeys. Soc. Neurosci. Abstr. 24, 1650.

Charara, A., Kulik, A., Shigemoto R., Smith, Y. (2000b) Cellular and subcellular localization of GABA-BR2 receptors in the basal ganglia in monkeys. Soc. Neurosci. Abstr. 26, 740.12

Chesselet, M.-F., Delfs, J.M. (1996) Basal ganglia and movement disorders: an update. Trends Neurosci.19, 417-422.

Cicchetti, F., Beach, T.G., Parent, A. (1998) Chemical phenotype of calretinin interneurons in the human striatum. Synapse 30, 284-297.

Cicchetti, F., Vinet, J., Beach, T.G., Parent, A. (1999) Differential expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor subunits by calretinin-immunoreactive neurons in the human striatum. Neuroscience 93, 89-97.

Ciliax, B.J., Greenamyre, T.J., Levey, A.I. (1997) Functional biochemistry and molecular neuropharmacology of the basal ganglia and motor systems. In Movement Disorders-Neurologic Principles and Practice (eds Watts, R.L. and Koller, W.C.) pp. 99-116, McGraw-Hill, New York.

Clarke, N.P., Bolam, J.P. (1998) Distribution of glutamate receptor subunits at neurochemically characterized synapses in the entopeduncular nucleus and subthalamic nucleus of the rat. J. Comp. Neurol. 397, 403-420.

Conn, P.J., Pin, J.P. (1997) Pharmacology and functions of metabotropic glutamate receptors. Ann. Rev. Pharmacol. Toxicol. 37, 205-237.

Counihan, T.J., Landwehrmeyer, G.B., Standaert, D.G., Kosinski, C.M., Scherzer, C.R., Daggett, L.P., Veliçelebi, G., Young, A.B., Penney, J.B., Jr. (1998) Expression of N-methyl-D-aspartate receptor subunit mRNA in the human brain: mesencephalic dopaminergic neurons. J. Comp. Neurol. 390, 91-101.

Davies, J. (1981) Selective depression of synaptic excitation in cat spinal neurones by baclofen: an iontophoretic study. Br. J. Pharmacol. 72, 373-384.

Davis, G.C., Williams, A.C., Markey, S.P., Ebert, M.H., Caine, E.D., Reichert, C.M., Kopin, I.J. (1979) Chronic parkinsonism secondary to intravenous injection of meperidine analogues. Psychiatry Res. 1, 249-254.

Deisz, R.A. (1997) Electrophysiology of GABAB receptors. In The GABA Receptors, Second Edition (eds. Enna, S.J. and Bowery, N.G.) pp. 157-207, Humana Press, Totowa, NJ.

DeLong, M.R. (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci. 13, 281-285.

Dubinsky, J.M. (1989) Development of inhibitory synapses among striatal neurons *in vitro*. J. Neurosci. 9, 3955-3965.

Dure, L.S., IV, Young, A.B., Penney, J.B. (1991) Excitatory amino acid binding sites in the caudate nucleus and frontal cortex of Huntington's disease. Ann. Neurol. 30, 785-793.

Dure, L.S., IV, Young, A.B., Penney, J.B., Jr. (1992) Compartmentalization of excitatory amino acid receptors in human striatum. Proc. Natl. Acad. Sci. USA 89, 7688-7692.

Dzubay, J.A., Jahr, C.E. (1999) The concentration of synaptically released glutamate outside of the climbing fiber-Purkinje cell synaptic cleft. J. Neurosci. 19, 5265-5274.

Enz, R., Brandstatter, J.H., Hartveit, E., Wassle, H., Bormann, J. (1995) Expression of GABA receptor rho 1 and rho 2 subunits in the retina and brain of the rat. Eur. J. Neurosci. 7, 1495-1501.

Enz, R., Cutting, G.R. (1999) $GABA_C$ receptor ρ subunits are heterogeneously expressed in the human CNS and form homo- and heterooligomers with distinct physical properties. Eur. J. Neurosci. 11, 41-50.

Faull, R.L., Villiger, J.W. (1986) Heterogeneous distribution of benzodiazepine receptors in the human striatum: a quantitative autoradiographic study comparing the pattern of receptor labelling with the distribution of acetylcholinesterase staining. Brain Res. 381, 153-158.

Faull, R.L., Villiger, J.W. (1988) Multiple benzodiazepine receptors in the human basal ganglia: a detailed pharmacological and anatomical study. Neuroscience 24, 433-451.

Fox, S., Krnjevic, K., Morris, M.E., Puil, E., Werman, P. (1978) Action of baclofen on mammalian synaptic transmission. Neuroscience 3, 495-515.

Fritschy, J.-M., Meskenaite, V., Weinmann, O., Honer, M., Benke, D., Möhler, H. (1999) GABAB-receptor splice variants GB1a and GB1b in rat brain: developmental regulation, cellular distribution and extrasynaptic localization. Eur. J. Neurosci. 11, 761-768.

Fritschy, J.-M., Möhler, H. (1995) $GABA_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J. Comp. Neurol. 359,154-194.

Froestl, W., Bettler, B., Bittiger, H., Heid, J., Kaupmann, K., Mickel, S., Strub, D. (1999) Ligands for the isolation of $GABA_B$ receptors. Neuropharmacology 38, 1641-1646.

Fujiyama, F., Fritschy, J.-M., Stephenson, F.A., Bolam, J.P. (2000) Synaptic localization of $GABA_A$ receptor subunits in the striatum of the rat. J. Comp. Neurol. 416, 158-172.

Fujiyama, F., Fritschy, J.-M., Stephenson, F.A., Bolam, J.P. (1998) Synaptic localization of GAB_A receptor subunits in the basal ganglia in the rat. Proceedings of the 6th International Basal Ganglia Society, p. 16, Brewster, MA.

Gasic, G.P., Hollmann, M. (1992) Molecular neurobiology of glutamate receptors. Annu. Rev. Physiol. 54, 507-536.

Gerfen, C.R., Enber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma, F.J., Jr., Sibley, D.R. (1990) D_1 and D_2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250, 1429-1432.

Gerfen, C.R., Wilson, C.J. (1996) The basal ganglia. In Handbook of Chemical Neuroanatomy, Integrated Systems of the CNS, Part III (eds. Björklund, A., Hökfelt, T. and Swanson, L.) pp. 369-466, Elsevier, Amsterdam.

Gerlach, M., Gsell, W., Kornhuber, J., Jellinger, K., Krieger, V., Pantucek, F., Vock, R., Riederer, P. (1996) A postmortem study on neurochemical markers of dopaminergic, GABA-ergic and glutamatergic neurons in basal ganglia-thalamocortical circuits in Parkinson syndrome. Brain Res. 741, 142-152.

Glass, M., Dragunow, M., Faull, R.L.M. (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and $GABA_A$ receptor alterations in the human basal ganglia in Huntington's disease. Neuroscience 97, 505-519.

Glaum, S.R., Miller, R.J. (1993) Activation of metabotropic glutamate receptors produces reciprocal regulation of ionotropic glutamate and GABA responses in the nucleus of the tractus solitarius of the rat J. Neurosci. 13, 1636-1641.

Glaum, S.R., Miller, R.J. (1994) Acute regulation of synaptic transmission by metabotropic glutamate receptors. In The Metabotropic Glutamate Receptors (eds. Conn, P.J. and Patel, J.) pp. 147-172, Humana Press, Totowa, NJ.

Grace, A.A., Bunney, B.S. (1984) The control of firing patternin nigral dopamine neurons: burst firing. J. Neurosci. 4, 2877-2890.

Graybiel, A.M. (1990) Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci. 13, 244-254.

Griffiths, P.D., Sambrook, M.A., Perry, R. Crossman, A.R. (1990) Changes in benzodiazepine and acetylcholine receptors in the globus pallidus in Parkinson's disease. J. Neurol. Sci. 100, 131-136.

Hanson, J.E., Smith, Y. (1999) Group I metabotropic glutamate receptors at GABAergic synapses in monkeys. J. Neurosci. 19, 6488-6496.

Healy, D.J., Haroutunian, V., Powchik, P., Davidson, M., Davis, K.L., Watson, S.J., Meador-Woodruff, J.H. (1998) AMPA receptor binding and subunit mRNA expression in prefrontal cortex and striatum of elderly schizophrenics. Neuropsychopharmacology 19, 278-286.

Heimer, L., Zahm, D.S., Alheid, G.F. (1995) Basal ganglia. In The Rat Nervous System, Second Edition (ed. Paxinos, G.) pp. 579-628, Academic Press, San Diego.

Helton, D.R., Tizzano, J.P., Monn, J.A., Schoepp, DD., Kallman, M.J. (1998) Anxiolytic and side-effect profile of LY354740: a potent, highly selective, orally active agonist for group II metabotropic glutamate receptors. J. Pharmacol. Exp. Ther. 284, 651-660.

Hill, D.R., Bowery, N.G. (1981) ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. Nature 290, 149-152.

Hollmann, M., Heinemann, S. (1994) Cloned glutamate receptors. Ann. Rev. Neurosci. 17, 31-108.

Hoover, J.E., Strick, P.L. (1999) The organization of cerebellar and basal ganglia outputs to primary motor cortex as revealed by retrograde transneuronal transport of herpes simplex virus type 1. J. Neurosci. 19, 1446-1463.

Hubert, G.W., Smith, Y. (1999) Subsynaptic localization of group I metabotropic glutamate receptors (mGluRs) in the ventral midbrain of rats. Soc. Neurosci. Abstr. 25, 1156.

Ilinsky, I.A., Jouandet, M.L., Goldman-Rakic, P.S. (1985) Organization of the nigrothalamocortical system in the rhesus monkey. J. Comp. Neurol. 236, 315-330.

Ilinsky, I.A., Tourtelotte, W.G., Kultas-Ilinsky, K. (1993) Anatomical distinctions between the two basal ganglia afferent territories in the primate motor thalamus. Stereotact. Funct. Neurosurg. 60, 62-69.

Inglis, W.L., Winn, P. (1995) The pedunculopontine tegmental nucleus: where the striatum meets the reticular formation. Prog. Neurobiol. 47, 1-29.

Isaacson, J.S., Solis, J.M., Nicoll, R.A. (1993) Local and diffusion synaptic actions of GABA in the hippocampus. Neuron 10, 165-175.

Jo, Y.-H., Schlichter, R. (1999) Synaptic corelease of ATP and GABA in cultured spinal neurons. Nat. Neurosci. 2, 241-245.

Joel, D., Weiner, I. (1994) The organization of the basal ganglia-thalamocortical circuits: open interconnected rather than closed segregated. Neuroscience 63, 363-379.

Joel, D., Weiner, I. (1997) The connections of the primate subthalamic nucleus: indirect pathways and the open-interconnected scheme of basal ganglia-thalamocortical circuitry. Brain Res. Rev. 23, 62-78.

Johnston, G.A.R. (1996) $GABA_A$ receptor pharmacology. Pharmacol. Ther. 69, 173-198.

Johnston, G.A.R. (1997) Molecular biology, pharmacology, and physiology of GABAC receptors. In The GABA Receptors, Enna, S.J. and Bowery, N.G. (eds), pp. 297-323. Humana Press, Totowa, NJ.

Jonas, P., Bischofberger, J., Sandkuhler, J. (1998) Corelease of two fast neurotransmitters at a central synapse. Science 281, 419-424.

Jones, K.A., Borowsky, B., Tamm, J.A., Craig, D.A., Durkin, M.M., Dai, M., Yao, W.J., Johnson, M., Gunwaldsen, C., Huang, L.Y., Tang, C., Shen, Q., Salon, J.A., Morse, K., Laz, T., Smith, K.E., Nagarathnam, D., Noble, S.A., Branchek, T.A., Gerald, C. (1998) $GABA_B$ receptors function as heteromeric assembly of the subunits $GABA_BR1$ and $GABA_BR2$. Nature 396, 674-679.

Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froesti, W., Beck, P., Mosbacher, J., Bischoff, S., Kulik, A., Shigemoto, R., Karschin, A., Bettler, B. (1998) $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. Nature 396, 683-687.

Kawaguchi, Y., Wilson, C.J., Augood, S.J., Emson, P.C. (1995) Striatal interneurones: Chemical, physiological and morphological characterization. Trends Neurosci. 18, 527-535.

Kerr, D.I., Ong, J. (1995) GABAB receptors. Pharmacol. Ther. 67, 187-246.

Kinoshita, A., Shigemoto, R., Ohishi, H., van der Putten, H., Mizuno, N. (1998) Immunohistochemical localization of metabotropic glutamate receptors, mGluR7a and mGluR7b, in the central nervous system of the adult rat and mouse: a light and electron microscopic study. J. Comp. Neurol. 393, 332-352.

Kieval, J.Z., Paré, J.-F., Smith, Y. (2000) Subcellular localization of kainate receptors in the monkey striatum. Soc. Neurosci. Abstr. 26, 741.15.

Kita, H., Kitai, S.T. (1991) Intracellular study of rat globus pallidus neurons: membrane properties and responses to neostriatal, subthalamic and nigral stimulation. Brain Res. 564 296-305.

Kosinski, C.M., Bradley, S.R., Conn, P.J., Levey, A.I., Landwehrmeyer, G.B., Penney, J.B., Jr., Young, A.B., Standaert, D.G. (1999) Localization of metabotropic glutamate receptor 7 mRNA and mGluR7a protein in the rat basal ganglia. J. Comp. Neurol. 415, 266-284.

Kosinski, C.M., Standaert, D.G., Counihan, T.J., Scherzer, C.R., Kerner, J.A., Daggett, L.P., Veliçelebi, G., Penney, J.B., Young, A.B., Landwehrmeyer, G.B. (1998) Expression of N-methyl-D-aspartate receptor subunit mRNAs in the human brain: striatum and globus pallidus. J. Comp. Neurol. 390, 63-74.

Kullmann, D.M., Asztely, F. (1998) Extrasynaptic glutamate spillover in the hippocampus: evidence and implications. Trends Neurosci 21, 8-14.

Kultas-Ilinsky, K., Leontiev, V., Whiting, P.J. (1998) Expression of 10 $GABA_A$ receptor subunit messenger RNAs in the motor-related thalamic nuclei and basal ganglia of *Macaca mulatta* studied with *in situ* hybridization histochemistry. Neuroscience 85, 179-204.

Kuner, R., Köhr, G., Grünewald, S., Eisenhardt, G., Bach, A., Kornau, H.C. (1999) Role of heteromer formation in $GABA_B$ receptor function. Science 283, 74-77.

Küppenbender, K.D., Standaert, D.G., Feuerstein, T.J., Penney, J.B., Jr., Young, A.B., Landwehrmeyer, G.B. (2000) Expression of NMDA receptor subunit mRNAs in neurochemically identified projection and interneurons in the human striatum. J. Comp. Neurol. 419, 407-421.

Langston, J.W., Ballard, P.A., Tetrud, J.W., Irwin, I. (1983) Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219, 979-980.

Lee, H., Choi, B.H. (1992) Density and distribution of excitatory amino acid receptors in the developing human fetal brain: a quantitative autoradiographic study. Exp. Neurol. 118, 284-290.

Levine, M.S., Hull, C.D., Buchwald, N.A. (1974) Pallidal and entopeduncular intracellular responses to striatal, cortical, thalamic, and sensory inputs. Exp. Neurol. 44, 448-460.

Levy, R, Hazrati, L.-N., Herrero, M.-T., Vila, M., Hassani, O.K., Mouroux, M., Ruberg, M., Asensi, H., Agid, Y., Féger, J., Obeso, J.A., Parent, A., Hirsch, E.C. (1997) Re-evaluation

of the functional anatomy of the basal ganglia in normal and parkinsonian states. Neuroscience 76, 335-343.

Liu, F., Wan, Q., Pristupa, Z.B., Yu, X.-M., Wang, Y.T., Niznik, H.B. (2000) Direct protein-protein coupling enables cross-talk between dopamine D5 and γ-aminobutyric acid A receptors. Nature 403, 274-280.

Lujan, R., Nusser, Z., Roberts, J.D.B., Shigemoto, R., Somogyi, P. (1996) Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. Eur. J. Neurosci. 8, 1488-1500.

MacDonald, M.E., Vonsattel, J.P., Shrinidhi, J., Couropmitree, N.N., Cupples, L.A., Bird, E.D., Gusella, J.F., Myers, R.H. (1999) Evidence for the GluR6 gene associated with younger onset age of Huntington's disease. Neurology 53, 1330-1332.

Macdonald R.L., Olsen R.W. (1994) $GABA_A$ receptor channels. Annu. Rev. Neurosci. 17, 569-602.

Makoff, A. (1999) Molecular cloning of human $GABA_BR1$ and its tissue distribution. Mol. Brain Res. 64 ,137-140.

Margeta-Mitrovic, M., Mitrovic, I., Riley, R.C., Jan, L.Y., Basbaum, A.I. (1999) Immunocytochemical localization of $GABA_B$ receptors in the rat central nervous system. J. Comp. Neurol. 405, 299-321.

Marsden, C.D. (1984) Function of the basal ganglia as revealed by cognitive and motor disorders in Parkinson's disease. Can. J. Neurol. Sci. 11, 129-135.

Martin, L.J., Blackstone, C.D., Huganir, R.L., Price, D.L. (1993) The striatal mosaic in primates: striosomes and matrix are differentially enriched in ionotropic glutamate receptor subunits. J. Neurosci. 13, 782-792.

Martin, S.C., Russek, S.J., Farb, D.H. (1999) Molecular identification of the human GABABR2: cell surface expression and coupling to adenylyl cyclase in the absence of GABABR1. Mol. Cell Neurosci. 13, 180-191.

Matsubara, A., Laake, J.H., Davanger, S., Usami, S.-I., Ottersen O.P. (1996) Organization of AMPA receptor subunits at a glutamate synapse: A quantitative immunogold analysis of hair cell synapses in the rat organ of Corti. J. Neurosci. 16, 4457-4467.

Mayeux, R., Chen, J., Mirabello, E., Marder, K., Bell, K., Dooneief, G., Cote, L., Stern, Y. (1990) An estimate of the incidence of dementia in idiopathic Parkinson's disease. Neurology 40, 1513-01517.

Mayeux, R., Denaro, J., Hemenegildo, N., Marder, K., Tang, M.X., Cote, L.J., Stern, Y. (1992) A population-based investigation of Parkinson's disease with and without dementia. Relationship to age and gender. Arch. Neurol. 49, 492-497.

Mehta, A.K., Ticku, M.K. (1999) An update on $GABA_A$ receptors. Brain Res. Rev. 29, 196-217.

Meng, S.Z., Obonai, T., Isumi, H., Takashima, S. (1997) A developmental expression of AMPA-selective glutamate receptor subunits in human basal ganglia. Brain Dev. 19, 388-392.

Meoni, P., Bunnemann, B.H., Kingsbury, A.E., Trist, D.G., Bowery, N.G. (1999) NMDA NR1 subunit mRNA and glutamate NMDA-sensitive binding are differentially affected in the striatum and pre-frontal cortex of Parkinson's disease patients. Neuropharmacology 38, 625-633.

Middleton, F.A., Strick, P.L. (2000) Basal ganglia and cerebellar loops: Motor and cognitive circuits. Brain Res. Rev. 31, 236-250.

Misgeld, U., Bijak, M., Jarolimek, W. (1995) A physiological role for GABAB receptors and the effects of baclofen in the mammalian central nervous system. Prog, Neurobiol. 46, 423-462.

Moghaddam, B., Adams, B.V. (1998) Reversal of phencyclidine effects by group II metabotropic glutaamte receptor agonists in rats. Science 281, 1349-

Möhler, H., Benke, D., Benson, J., Lüscher, B., Rudolph, U., Fritschy, J.-M. (1997) Diversity in structure, pharmacology, and regulation of GABAA receptors. In The GABA Receptors, Second Edition (eds Enna, S.J. and Bowery, N.G.), pp. 1-36, Humana Press, Totowa, NJ.

Moerlein, S.M., Perlmutter, J.S. (1992) Binding of 5-(2'-[18F]fluoroethyl) flumazenil to central benzodiazepine receptors measured in living baboon by positron emission tomography. Eur. J. Pharmacol. 218, 109-115.

Morishita, W., Kirov, S.A., Alger, B.E. (1998) Evidence for metabotropic glutamate receptor activation in the induction of depolarization-induced supprression of inhibition in hippocampal CA1. J. Neurosci. 18, 4870-4882.

Muriel, M.P., Bernard, V., Levey, A.I., Laribi, O., Abrous, D.N., Agid, Y., Bloch, B., Hirsch, E. (1999) Levodopa induces a cytoplasmic localization of D1 dopamine receptors in striatal neurons in Parkinson's disease. Ann. Neurol. 46, 103-111.

Nakanishi, S. (1994) Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity. Neuron 13, 1031-1037.

Neale, R., Gerhardt, S., Liebman, J.M. (1984) Effects of dopamine agonists, catecholamine depletors, and cholinergic and GABAergic drugs on acute dyskinesia in squirrel monkeys. Psychophramacol. 82, 20-26.

Nisenbaum, E.S., Berger, T.W., Grace, A.A. (1992) Presynaptic modulation by $GABA_B$ receptors of glutamatergic excitation and GABAergic inhibition of neostriatal neurons. J. Neurophysiol. 67, 477-481.

Nisenbaum, E.S., Berger, T.W., Grace, A.A. (1993) Depression of glutamatergic and GABAergic synaptic responses in striatal spiny neurons by stimulation of presynaptic GABAB receptors. Synapse 14, 221-242.

Noga, J.T., Hyde, T.M., Herman, M.M., Spurney, C.F., Bigelow, L.B., Weinberger, D.R., Kleinman, J.E. (1997) Glutamate receptors in the postmortem striatum of schizophrenic, suicide, and control brains. Synapse 27, 168-176.

Nusser, Z. (1999) A new approach to estimate the number, density and variability of receptors at central synapses. Eur. J. Neurosci. 11, 745-752.

Nusser, Z., Cull-Candy, S., Farrant, M. (1997) Differences in synaptic $GABA_A$ receptor number underlie variation in GABA mini amplitude. Neuron 19, 697-709.

Nusser, Z., Mulvihill, E., Streit, P., Somogyi, P. (1994) Subsynaptic segregation of metabotropic and ionotropic glutamate receptors as revealed by immunogold localization. Neuroscience 61, 421-427.

Nusser, Z., Roberts, J.D.B., Baude, A., Richards, J.G., Somogyi, P. (1995) Relative densities of synaptic and extrasynaptic $GABA_A$ receptors on cerebellar granule cells as determined by a quantitative immunogold method. J. Neurosci. 15, 2948-2960.

Nusser, Z., Sieghart, W., Somogyi, P. (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J. Neurosci. 18, 16939-1703.

Ohishi, H., Shigemoto, R., Nakanishi, S., Mizuno, N. (1993) Distribution of the messenger RNA for a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat. Neuroscience 53, 1009-1018.

Ohishi, H., Akazawa, C., Shigemoto, R., Nakanishi, S., Mizuno, N. (1995) Distributions of the mRNAs for L-2-amino-4-phosphonobutyrate-sensitive metabotropic glutamate receptors, mGluR4 and mGluR7, in the rat brain. J. Comp. Neurol. 360, 555-570.

Ohye, C., Kimura, M., McKenzie, J.S. (1996) The Basal Ganglia V. 519 pp. Plenum Press, New York.

Olpe, H.R., Karlsson, G., Pozzza, M.E., Brugger, F., Steinmann, M., Van Riezen, H., Fagg, G., Hall, R.G., Froestl, W., Bittiger, H. (1990) CGP 35348: a centrally active blocker of GABAB receptors. Eur. J. Pharmacol. 187, 27-38.

Ong, J., Marino, V., Parker, D.A.S., Kerr, D.I.B., Froestl, W. (1999) Pharmacological re-evaluation of a $GABA_B$ receptor antagonist CGP 47332A in rat brain. Eur. J. Pharmacol. 383, 137-141.

Ottersen, O.P., Landsend, A.S. (1997) Organization of glutamate receptors at the synapse. Eur. J. Neurosci. 9, 2219-2224.

Paquet, M., Smith, Y. (1996) Differential localization of AMPA glutamate receptor subunits in the two segments of the globus pallidus and the substantia nigra pars reticulata in the squirrel monkey. Eur. J. Neurosci. 8, 229-233.

Paquet, M., Smith, Y. (1997) Differential distribution of metabotropic glutamate receptors (mGluRs) in the monkey striatum. Soc. Neurosci. Abstr. 23, 188.

Paquet, M., Tremblay, M., Soghomonian, J.-J., Smith, Y. (1997) AMPA and NMDA glutamate receptor subunits in midbrain dopaminergic neurons in the squirrel monkey: an immunohistochemical and *in situ* hybridization study. J. Neurosci, 17, 1377-1396.

Parent, A., Hazrati, L.-N. (1995) Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res. Rev. 20, 91-127.

Parpura, V., Basarsky, T.A., Liu, F., Jeftinija, S., Haydon, P.G. (1994) Glutamate-mediated astrocyte-neuron signaling. Nature 369, 744-747.

Partenain, A.V., Morales, M., Lerma, J. (1995) Selective antagonism of AMPA receptors unmasks kainate receptor-mediated responses in hippocampal neurons. Neuron 14, 185-189.

Penney, J.B., Young, A.B. (1982) Quantitative autoradiography of neurotransmitter receptors in Huntington's disease. Neurology 32, 1391-1395.

Percheron, G., McKenzie, J.S., Féger, J. (1994) The Basal Ganglia IV. 616 pp. Plenum Press, New York.

Petralia, R.S., Wang, Y.-X., Wenthold, R.J. (1994a) Histological and ultrastructural localization of the kainate receptor subunits, KA2 and GluR6/7, in the rat nervous system using selective antipeptide antibodies. J. Comp. Neurol. 349, 85-110.

Petralia, R.S., Wenthold R.J. (1992) Light and electron immunocytochemical localization of AMPA-selective glutamate receptors in the rat brain. J. Comp. Neurol. 318, 329-354.

Petralia, R.S., Yokotani, N., Wenthold, R.J. (1994b) Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. J. Neurosci. 14, 667-696.

Phillips, T., Rees, S., Augood, S., Waldvogel, H., Faull, R., Svendsen, C., Emson, P. (2000) Localization of metabotropic glutamate receptor type 2 in the human brain. Neuroscience 95, 1139-156.

Pin, J.-P., Duvoisin, R. (1995) Neurotransmitter receptors I. The metabotropic glutamate receptors: Structure and functions. Neuropharmacology 34, 1-26.

Poewe, W., Granata, R. (1997) Pharmacological treatment of Parkinson's disease. In Movement Disorders-Neurologic Principles and Practice. Watts, R.L. and Koller, W.C. (eds), pp. 201-220. McGraw-Hill, New York.

Porter, B. (1997) A review of intrathecal baclofen in the management of spasticity. Br. J. Nurs. 6, 253-

Porter, J.T., McCarthy, K.D. (1999) Astrocytic neurotransmitter receptors in situ and in vivo. Prog. Neurobiol. 51, 439-455.

Reinmann, W. (1983) Inhibition by GABA, baclofen and gabapentin of dopamine release from rabbit caudate nucleus. Are there common or different sites of action? Eur. J. Pharmacol. 94, 341-344.

Robertson, R.G., Clarke, C.E., Boyce, S., Sambrook, M.A., Crossman, A.R. (1989) GABA/benzodiazepine receptors in the primate basal ganglia following treatment with MPTP: evidence for the differential regulation of striatal output by dopamine? In Neural Mechanisms in Disorders of Movement (eds. Crossman, A.R. and Sambrook, M.A.), John Libbey, London.

Robertson, R.G., Clarke, C.E., Boyce, S., Sambrook, M.A., Crossman, A.R. (1990) The role of striatopallidal neurones utilizing gamma-aminobutyric acid in the pathophysiology of MPTP-induced parkinsonism in the primate: evidence from [^{3}H]flunitrazepam autoradiography. Brain Res. 531, 95-104.

Rouillier, E.M., Liang, F., Babalian, A., Moret, V., Wiesendanger, M. (1994) Cerebellothalamocortical and pallidothalamocortical projections to the primary and

supplementary motor cortical areas: A multiple tracing study in macaque monkeys. J. Comp. Neurol. 345, 185-213.

Rubinztein, D.C., Leggo, J., Chiano, M., Norbury, G., Rosser, E., Craufurd, D. (1997) Genotypes of the GluR6 kainate receptor locus are associated with variation in the age of onset of Huntington's disease. Proc. Natl. Acad. Sci. (USA) 94, 3872-3876.

Rye, D.B. (1997) Contributions of the pedunculopontine region to normal and altered REM sleep. Sleep 20, 757-788.

Sano, M., Stern, Y., Williams, J., Cote, L., Rosenstein, R., Mayeux, R. (1989) Coexisting dementia and depression in Parkinson's disease. Arch. Neurol. 46, 1284-1286.

Sadikot, A.F., Smith, Y., Bolam, J.P., Parent, A. (1992a) Efferent connections of the centromedian and parafascicular thalamic nuclei in the squirrel monkey: a light and electron microscopic study of the thalamostriatal projection in relation to striatal heterogeneity. J. Comp. Neurol. 320, 228-242.

Sadikot, A., Parent, A. François, C. (1992b) Efferent connections of the centromedian and parafascicular thalamic nuclei in the squirrel monkey: a PHA-L study of subcortical projections. J. Comp. Neurol. 315, 137-159.

Sawynok, J., LaBella, F.S. (1981) GABA and Baclofen potentiate the K^+-evoked release of methionine-enkephalin from rat slices. Eur. J. Pharmacol. 70, 103-110.

Schmid, L., Bottlaender, M., Fuseau, C., Fournier, D., Brouillet, E., Maziere, M. (1995) Solpidem displays heterogeneity in its binding to the nonhuman primate benzodiazepine receptor in vivo. J. Neurochem. 65, 1880-1886.

Schoepp, D.D., Jane, D.E., Monn, J.A. (1999) Pharmacological agents acting at subtypes of metabotropic glutamate receptors. Neuropharmacology 38, 1431-1476.

Seabrook, G.R., Howson, W., Lacey, M.G. (1990) Electrophysiological characterization of potent agonists and antagonists at pre- and postsynaptic $GABA_B$ receptors in rat brain slices. Br. J. Pharmacol. 101, 949-957.

Shen, K.Z., Johnson, S.W. (1997) Presynaptic GABAB and adenosine A1 receptors regulate synaptic transmission to rat substantia nigra reticulata neurones. J. Physiol. 505, 153-163.

Shink, E., Smith, Y. (1995) Differential synaptic innervation of neurons in the internal and external segments of the globus pallidus by the GABA- and glutamate-containing terminals in the squirrel monkey. J. Comp. Neurol. 358, 119-141.

Shink, E., Sidibé, M., Smith, Y. (1997) Efferent connections of the internal globus pallidus in the squirrel monkey: II. Topography and synaptic organization of pallidal efferents to the pedunculopontine nucleus. J. Comp. Neurol. 382, 348-363.

Sidibé, M., Bevan, M.D., Bolam, J.P., Smith, Y. (1997) Efferent connections of the internal globus pallidus in the squirrel monkey: I. Topography and synaptic organization of the pallidothalamic projection. J. Comp. Neurol. 382, 323-347.

Sidibé, M., Smith, Y. (1999) Thalamic inputs to striatal interneurons in monkeys: Synaptic organization and co-localization of calcium binding proteins. Neuroscience 89, 1189-1208.

Smith, Y., Bennett, B.D., Bolam, J.P., Parent, A., Sadikot, A.F. (1994) Synaptic relationships between dopaminergic afferents and cortical or thalamic input in the sensorimotor territory of the striatum in monkey. J. Comp. Neurol. 344, 1-19.

Smith, Y., Bevan, M.D., Shink, E., Bolam, J.P. (1998a) Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 86, 353-387.

Smith, A.D. and Bolam, J.P. (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci. 13, 259-265.

Smith, Y., Charara, A., Hanson, J.E., Paquet, M., Levey, A.I. (2000a) $GABA_B$ and group I metabotropic glutamate receptors in the striatopallidal complex in primates. J. Anat. 196, 555-576.

Smith, I.D., Grace, A.A. (1992) Role of the subthalamic nucleus in regulation of nigral dopamine neuron activity. Synapse 12, 287-303.

Smith, Y., Parent, A. (1986) Differential connections of caudate nucleus and putamen in the squirrel monkey (*Saimiri sciureus*). Neuroscience 18, 347-371.

Smith, Y., Sidibé, M., Paré, J.-F. (2000b) Synaptic inputs from the substantia nigra and the pedunculopontine nucleus to thalamostriatal neurons in monkeys. Soc. Neurosci. Abst. 26, 361.15.

Smith, Y., Sidibé, M. (1999) Functional interconnections between the caudal intralaminar nuclei and the striatopallidal complex in monkeys. Soc. Neurosci. Abstr. 25, 884.19.

Smith, Y., Shink, E., Sidibé, M. (1998B) Neuronal circuitry and synaptic connectivity of the basal ganglia. Neurosurg. Clin. N. Am. 9, 203-222.

Smolders, I., De Klippel, N., Sarre, S., Ebinger, G., Michotte, Y. (1995) Tonic GABAergic modulation of striatal dopamine release studied by in vivo microdialysis in the freely moving rat. Eur. J. Pharmacol. 284, 83-91.

Sontheimer, H., Black, J.A., Waxman, S.G. (1996) Voltage-gated Na^+ channels in glia: properties and possible functions. Trends Neurosci. 19, 325-331.

Standaert, D.G., Testa, C.M., Young, A.B., Penney, J.B., Jr. (1994) Organization of N-methyl-D-aspartate glutamate receptor gene expression in the basal ganglia of the rat. J. Comp. Neurol. 343, 1-16.

Starr, M.S. (1995) Glutamate/dopamine D1/D2 balance in the basal ganglia and its relevance to Parkinson's disease. Synapse 19, 264-293.

Steinhauser, C., Gallo, V. (1996) News on glutamate receptors in glial cells. Trends Neurosci. 19, 339-345.

Svingos, A.L., Moriwaki, A., Wang, J.B., Uhl, G.R., Pickel, V.M. (1997) Mu-opioid receptors are localized to extrasynaptic plasma membranes of GABAergic neurons and their targets in the rat nucleus accumbens. J. Neurosci. 17, 2585-2594

Testa, C.M., Friberg, I.K., Weiss, S.W., Standaert, D.G. (1998) Immunohistochemical localization of metabotropic glutamate receptors mGluR1a and mGluR2/3 in the rat basal ganglia. J. Comp. Neurol. 390, 5-19.

Testa, C.M., Standaert, D.G., Young, A.B., Penney, JB., Jr. (1994) Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. J. Neurosci. 14, 3005-3018.

Tomiyama, M., Palacios, J.M., Cortés, R., Vilaró, M.T., Mengod, G. (1997) Distribution of AMPA receptor subunit mRNAs in the human basal ganglia: an *in situ* hybridization study. Mol. Brain Res. 46, 281-289.

Ulas, J., Weihmuller, F.B., Brunner, L.C., Joyce, J.N., Marshall, J.F., Cotman, C.W. (1994) Selective increase of NMDA-sensitive glutamate binding in the striatum of Parkinson's disease, Alzheimer's disease, and mixed Parkinson's disease/ Alzheimer's disease patients: an autoradiographic study. J. Neurosci. 14(11 Pt. 1), 6317-6324.

Upton, N., Blackburn, T. (1997) Pharmacology of mammalian $GABA_A$ receptors. In The GABA Receptors. Enna, S.J. and Bowery, N.G. (eds), pp. 83-120. Humana Press, Totowa N.J.

Verkhratsky, A., Kettenmann, H. (1996) Calcium signalling in glial cells. Trends Neurosci. 19, 346-351.

Vitek, J.L. (1997) Stereotaxic surgery and deep brain stimulation for Parkinson's disease and movement. In Movement Disorders-Neurologic Principles and Practice. Watts, R.L. and Koller, W.C. (eds), pp. 237-256. McGraw-Hill, New York.

Vonsatell, J.P.G., DiFiglia, M. (1998) Huntington's disease. J. Neuropathol. Exp. Neurol. 57, 369-384.

Waldvogel, H.J., Fritschy, J.-M., Mohler, H., Faull, R.L.M. (1998) $GABA_A$ receptors in the primate basal ganglia: an autoradiographic and a light and electron microscopic immunohistochemical study of the $\alpha 1$ and $\beta 2,3$ subunits in the baboon brain. J. Comp. Neurol. 397, 297-325.

Waldvogel, H.J., Kubota, Y., Fritschy, J.-M., Mohler, H., Faull, R.L.M. (1999) Regional and cellular localisation of $GABA_A$ receptor subunits in the human basal ganglia: an autoradiographic and immunohistochemical study. J. Comp. Neurol. 415, 313-340.

Walker F.O., Young, A.B., Penney, J.B., Dovorini-Kis, K., Shoulson, I. (1984) Benzodiazepine and GABA receptors in early Huntington's disease. Neurology 34, 1237-1240.

Wegelius, K., Pasternack, M., Hiltunen, J.O., Rivera, C., Kaila, K., Saarma, M., Reeben, M. (1998) Distribution of GABA receptor rho subunit transcripts in the rat brain. Eur. J. Neurosci. 10, 350-357.

Westbrook, G.L. (1994) Glutamate receptor update. Curr. Opin. Neurobiol. 4, 337-346.

White, L.E., Hodges, H.D., Carnes, K.M., Price, J.L., Dubinsky, J.M. (1994) Colocalization of excitatory and inhibitory neurotransmitter markers in striatal projection neurons in the rat. J. Comp. Neurol. 339, 328-340.

White, J.H., Wise, A., Main, M.J., Green, A., Fraser, N.J., Disney, G.H., Barnes, A.A., Emson, P., Foord, S.M., Marshall, F.H. (1998) Hetrodimerization is required for the formation of a functional GABAB receptor. Nature 396, 679-682.

Wichmann, T., DeLong, M.R. (1996) Functional and pathophysiological models of the basal ganglia. Curr. Opin. Neurobiol. 6, 751-758.

Wilson, C.J. (1998) Basal ganglia. In The Synaptic Organization of the Brain, Fourth Edition (ed. Shepherd, G.M.) pp. 329-375. Oxford University Press, New York.

Wilson, J.S., Wilson, J.A. (1985) Baclofen attenuates hyperpolarizing not depolarizing responses of caudate neurons in cat. Brain Res. 342, 396-400.

Wisden, W., Laurie, D.J., Monyer, H., Seeburg, P.H. (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J. Neurosci. 12, 1040-1062

Young, W., Kuhar, M.J. (1979) Autoradiographic localization of benzodiazepine receptors in the brains of humans and animals. Nature 280, 393-395.

Yoon, K.-W., Rothman, S.M. (1991) The modulation of rat hippocampal synaptic conductances by baclofen and γ-aminobutyric acid. J. Physiol. 442, 377-390.

Yung, K.K.L., Bolam, J.P., Smith, A.D., Hersch, S.M., Ciliax, B.J., Levey, A.I. (1995) Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: Light and electron microscopy. Neuroscience 65, 709-730.

Zhang, J.-H., Sato, M., Tohyama, M. (1991) Region-specific expression of the mRNAs encoding β subunits (β1, β2 and β3) of $GABA_A$ receptor in the rat brain. J. Comp. Neurol. 303, 637-657.

FIGURE LEGEND

FIG.1: Connectivity of the Primate Basal Ganglia. Schematic representation of the main afferent and efferent connections of basal ganglia structures in primates. For the sake of clarity, some connections have been omitted. Abbreviations: CD: caudate nucleus; CM/Pf: Centre median/parafascicular complex; CTX: cerebral cortex; DR: dorsal raphe; GPe: globus pallidus, external segment; GPi: globus pallidus, internal segment; Hb: habenular nucleus; PUT: putamen; RF: reticular formation; Rt: reticular nuclesu; SC: superior colliculus; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; STN: subthalamic nucleus; Th: thalamus.

FIG. 2: Kainate Receptor Subunits in the Striatum. (A) GluR6/7-immunoreactive elements in the monkey caudate nucleus. Immunoreactivity is mainly associated with dendrites (DEN) and axon terminals (Te) forming asymmetric synapses (arrowheads) whereas spines (SP) are almost completely devoid of labelling. Non-immunoreactive terminals are indicated with asterisks. (B) GluR6/7-immunoreactive terminal (Te) forming an asymmetric axo-spinous synapse (arrowhead). Asterisks indicate non-immunoreactive terminals. Note the paucity of gold particles on the plasma membrane in the labelled bouton. (C) An anterogradely labelled terminal which displays GluR6/7 immunoreactivity (Te) in the monkey putamen following BDA injections in the primary motor cortex. The double labelled bouton forms an asymmetric synapse (arrowhead) with an unlabelled dendrite (DEN). An unlabeled terminal is marked in the neuropil (asterisk). (D-E) Post-embedding immunogold localization of GluR6/7 immunoreactivity in the monkey putamen. In immunoreactive terminals, gold particles are associated with vesicular membrane (double arrows in D) or aggregated in the presynaptic grid of asymmetric synapses (E). The postsynaptic immunoreactivity is often associated with the postsynaptic density (double arrows) and plasma membrane of asymmetric synaptic junctions (F). Scale bars: A: 1 μm; B: 0.5 μm (valid for C); D: 0.3 μm (valid for F); E: 0.5 μm.

FIG. 3: Group I mGluRs in the Monkey Striatum. (A) mGluR5 immunoreactivity (arrows) at the edges of a symmetric axo-dendritic synapse established by a TH-positive terminal in the putamen. (B) mGluR1a immunoreactivity (arrow) at the edges of an asymmetric axo-spinous synapse (arrowhead) in the caudate nucleus. (C) Summary diagram of the subsynaptic localization of mGluR1a and mGluR5 immunoreactivities in dendrites and spines of striatal projection neurons in monkeys. (D) mGluR1a immunoreactivity (arrows) in the main body of a symmetric axo-dendritic synapse in the caudate nucleus. Abbreviations: DA: dopamine; DEN: Dendrite; Glu: Glutamate; SNc: Substantia nigra pars compacta; SP: Spines; Thal: Thalamus. Scale bars: A: 0.25 μm (valid for D); B: 0.5 μm.

FIG. 4: GABA-BR1 Receptor Immunoreactivity in the Monkey Striatum. (A) GABA-BR1-immunoreactive terminal forming an symmetric axo-spinous synapse (arrowhead) in the monkey putamen. An immunoreactive dendrite (Den) is in the same field. (B) GABA-BR1 (GBR1) immunogold labeling (arrows) in the presynaptic grid of a putative glutamatergic terminal (asterisk) forming an asymmetric synapse (arrowhead) with a spine (SP) in the putamen. (C) Postsynaptic GABA-BR1 labelling (arrows) at the edges of an asymmetric axo-spinous synapse (arrowhead). (D) Postsynaptic GABA-BR1 labeling (arrow) at a symmetric axo-dendritic synapse. (E) Schematic diagram to summarize the subsynaptic localization of GABA-BR1 immunoreactivity in dendrites and spines of striatal projection neurons. Scale bars:

FIG. 5: Group I mGluR Immunoreactivity in the Monkey Pallidum. (A) MGluR1a immunogold labelling (arrows) in the main body of a symmetric axo-dendritic synapses established by striatal-like boutons (asterisks) in GPi. (B) Perisynaptic mGluR5 labelling (arrows) at a putative subthalamopallidal asymmetric synapse (arrowhead). (C) Summary diagram to illustrate the subsynaptic localization of group I mGluRs in the monkey pallidum. Note that group I mGluRs are expressed at both symmetric and asymmetric synapses. A substantial proportion of gold labelling was also found extrasynaptically. Scale bars: A: 0.3 μm; B: 0.6μm.

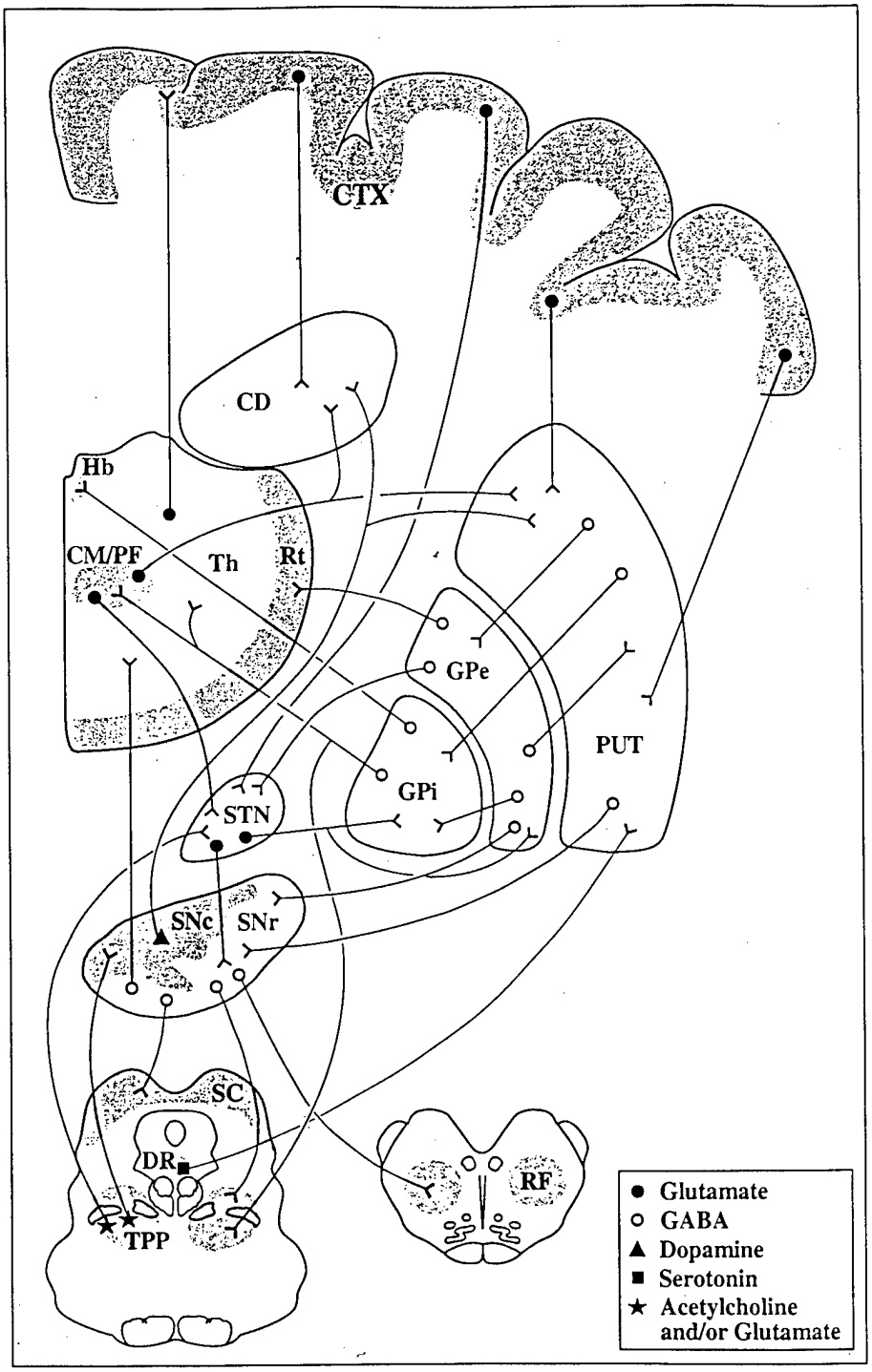
FIG. 6: GABA-A and GABA-B Receptors in the Monkey Pallidum. (A) GABA-A receptor $\alpha 1$ subunit immunoreactivity in the main body of a symmetric synapse (small arrows) established by a striatal-like bouton (asterisk). (B) Postsynaptic GABA-BR1 immunoreactivity (small arrows) at symmetric synapses established by putative striatal boutons (asterisks). (C) Postsynaptic labelling (arrows) at the edges of an asymmetric synapse estalished by a STN-like bouton in GPi (asterisk). (D-F) Presynaptic immunogold labelling of a STN-like bouton in GPi. Note that some gold particles are located in the presynaptic grid of the asymmetric synapse (arrows), but the majority of labelling is intracellular. Scale bars: A: 0.3 μm; B: 0.5 μm (valid for C-F).

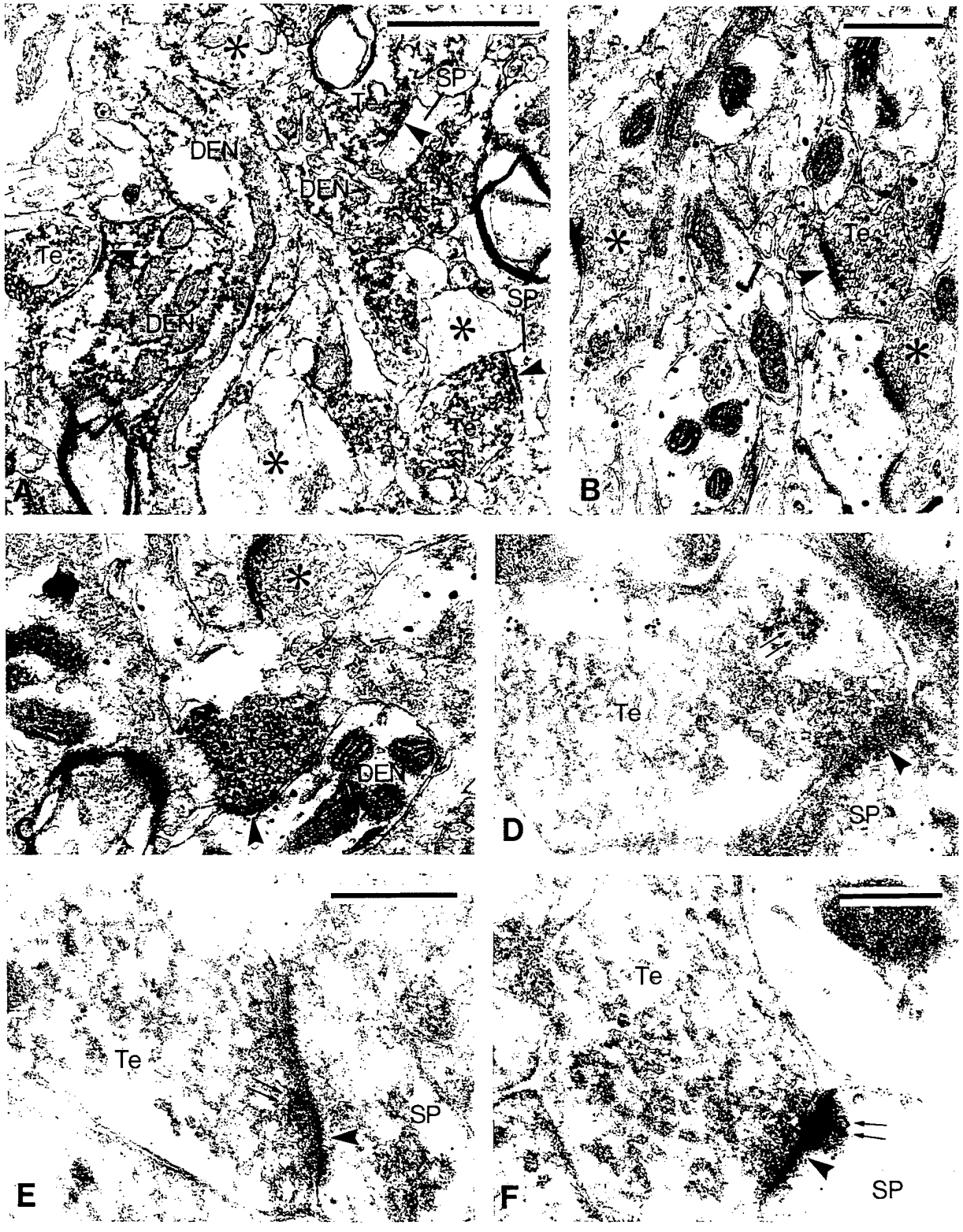
FIG. 7: Summary diagram to illustrate the subsynaptic localization of GABA-A and GABA-BR1 immunoreactivity in the monkey pallidum.

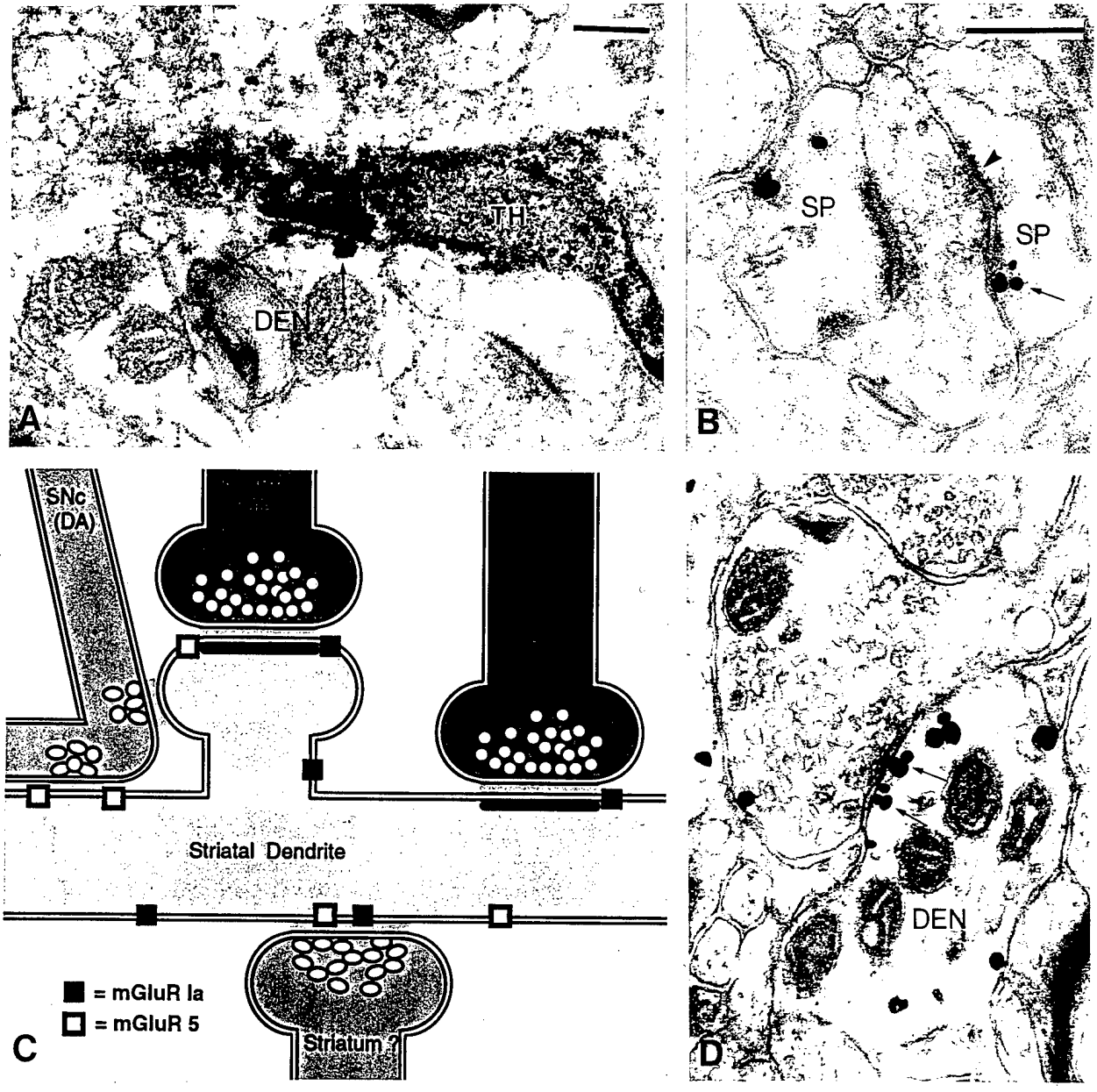
FIG. 8: Group I mGluRs and GABA-B Receptor Immunoreactivity in the STN. (A) mGluR1a labelling (small arrows) at the edges of a symmetric axo-dendritic synapse (arrows). (B) mGluR1a immunoreactivity at a "en passant" type symmetric synapse established by a vesicle-filled axon-like process (AX). Aggregates of gold particles are also found at extrasynaptic sites along the labelled dendrite. (C-D) mGluR1a immunoreactivity at the edges of an asymmetric postsynaptic specialization (C) or a puncta adherentia between a putative GABAergic GPe terminal and a dendrite (D). (E) A GABA-BR1-immunoreactive terminal forming asymmetric synapses (arrowheads) with dendrites (DEN). Scale bars: A: 0.2 μm (valid for B-E).

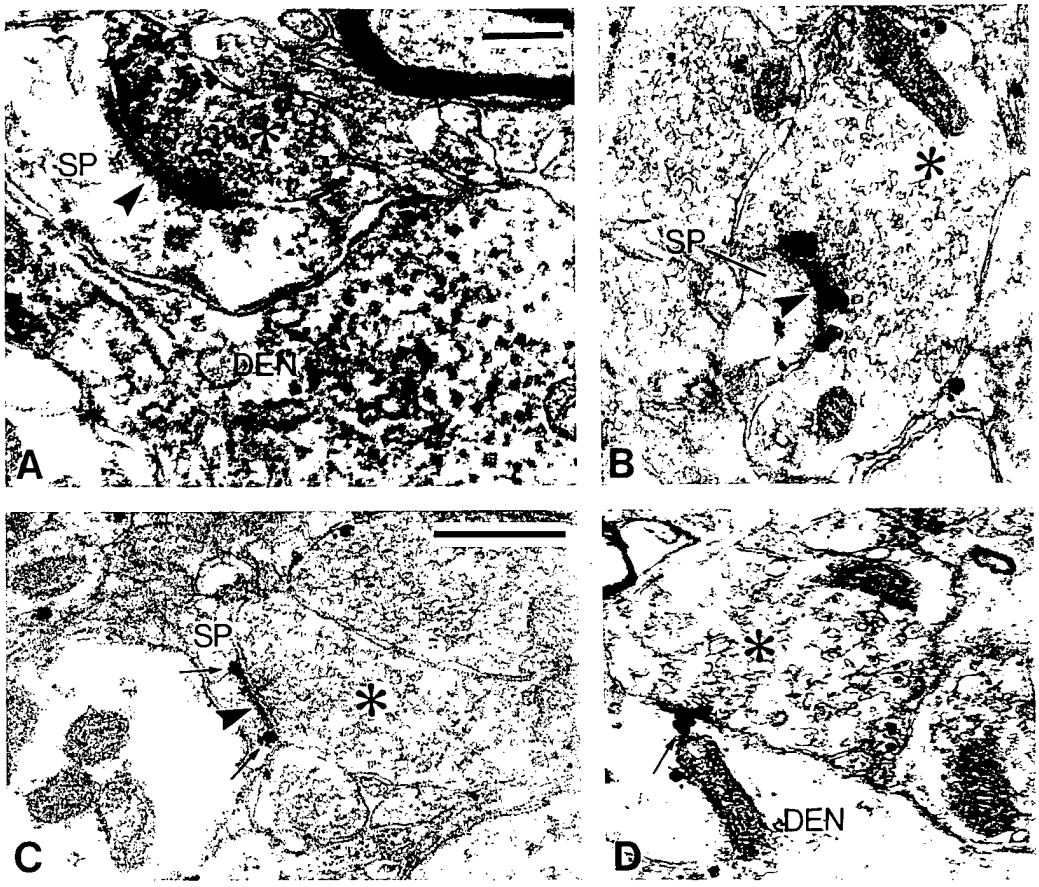
FIG. 9: Group I mGluRs and GABA-B Receptors in monkey SNc and SNr. (A) MGluR1a immunogold particles at the edges of asymmetric synapses on a small spine-like process. (B) MGluR1a labelling in the main body of a symmetric axo-dendritic synapse. (C) A GABA-BR1-immunoreactive axon terminal forming an asymmetric synapse with a labelled dendrite. The asterisk indicates an unlabeled bouton in contact with the same dendrite. (D) A GABA-BR1-

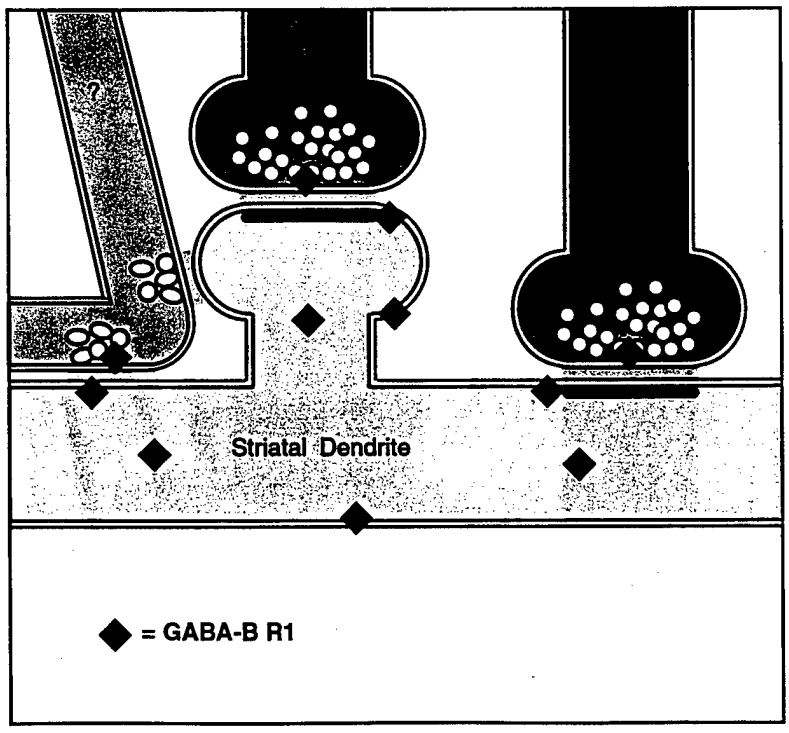
containing terminal in asymmetric contact with an immunoreactive dendrite in the SNr. The asterisk indicates a nonimmunoreactive terminal in contact with the same dendrite. Scale bars: A: 0.25 μm (valid for B-C); D: 0.5 μm.


CTX
CD
Hb
CM/PF
Th
Rt
GPe
PUT
GPi
STN
SNc
SNr
SC
DR
TPP
RF
Glutamate
GABA
Dopamine
Serotonin
Acetylcholine and/or Glutamate

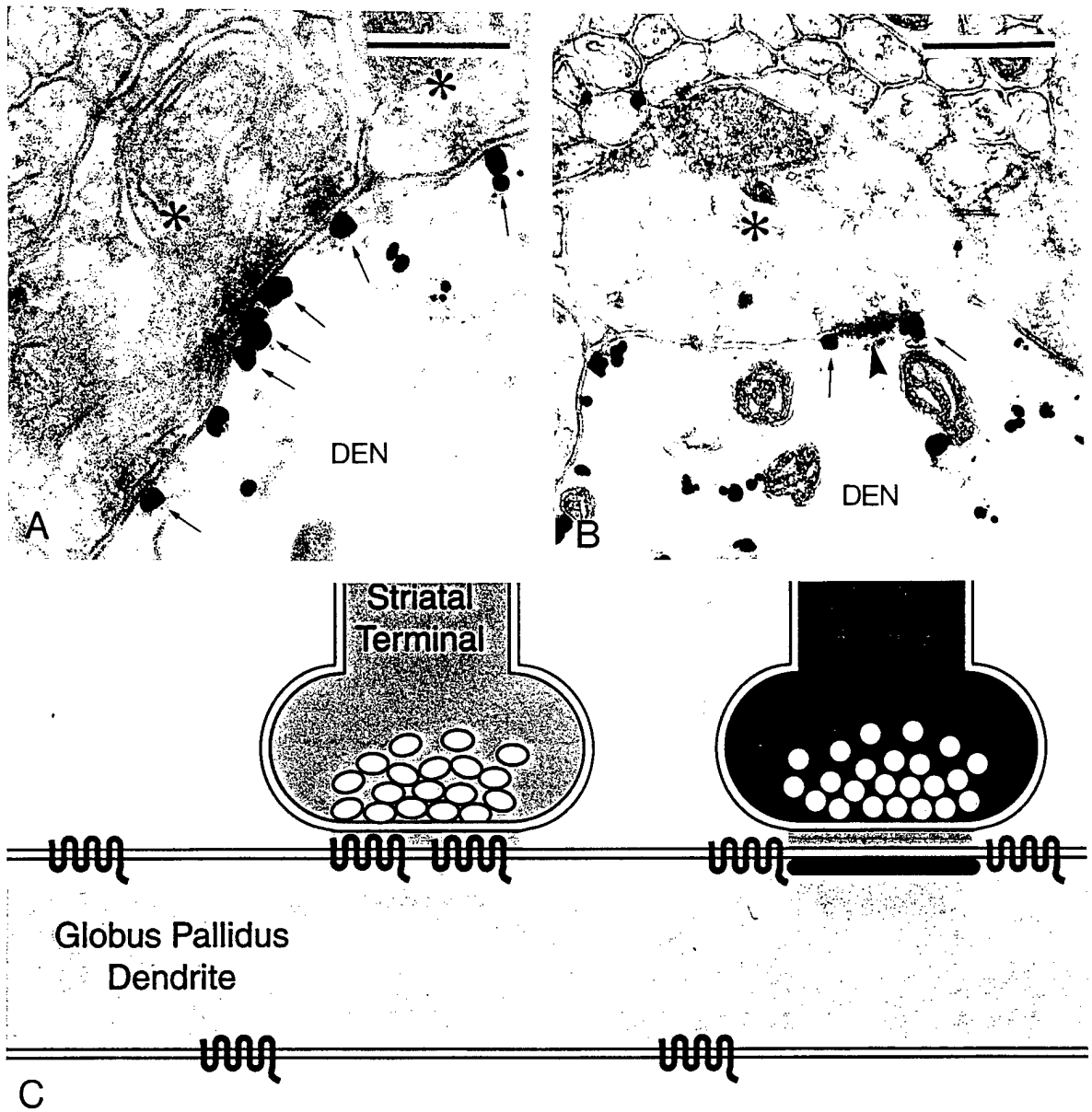

A
DEN
DEN
DEN
Te
Te
Te
SP
SP
B
Te
C
DEN
D
Te
SP
E
Te
SP
F
Te
SP

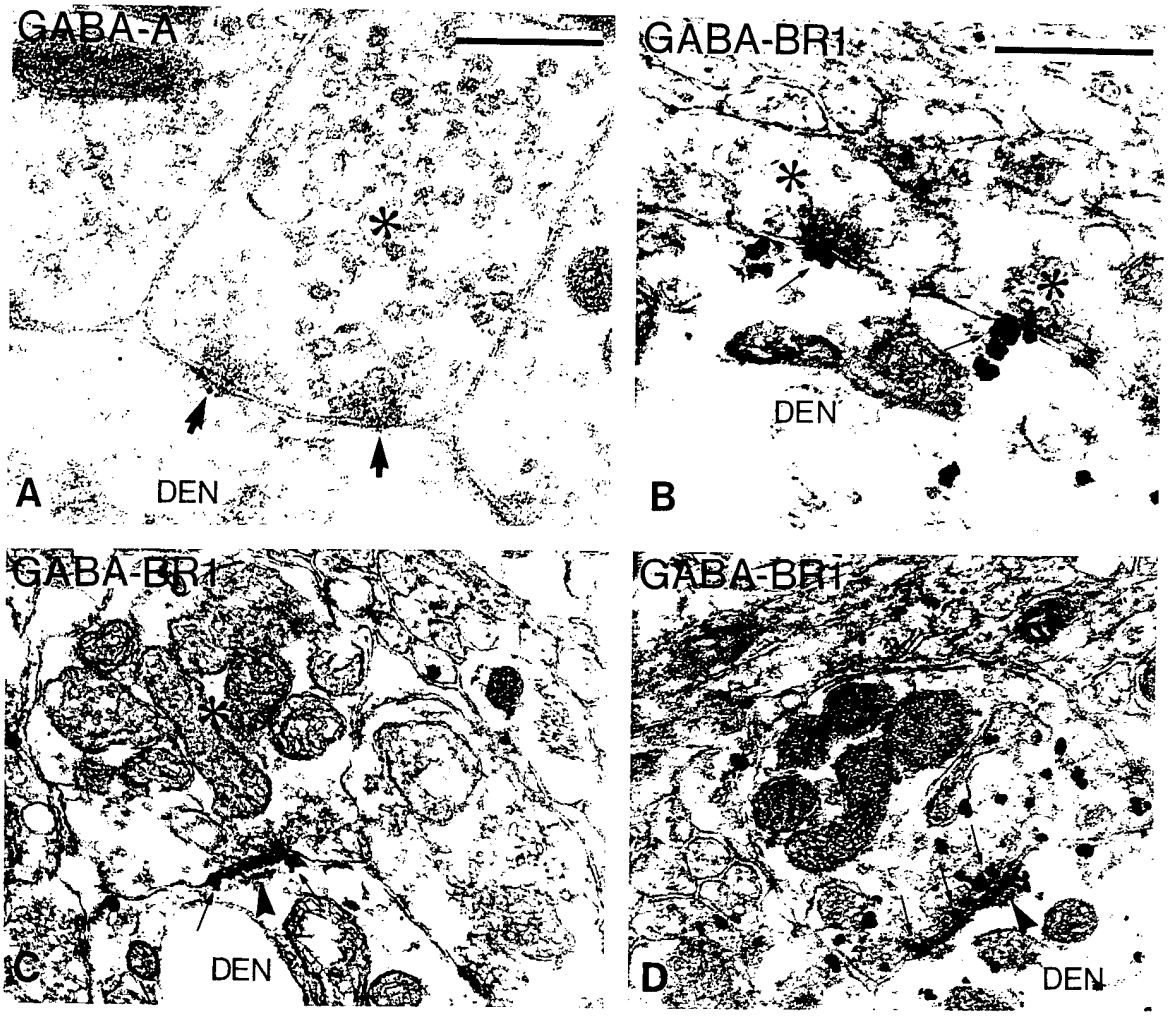

TH
DEN
A
SP
SP
B
SNc
(DA)
Striatal Dendrite
= mGluR Ia
= mGluR 5
Striatum ?
C
DEN
D

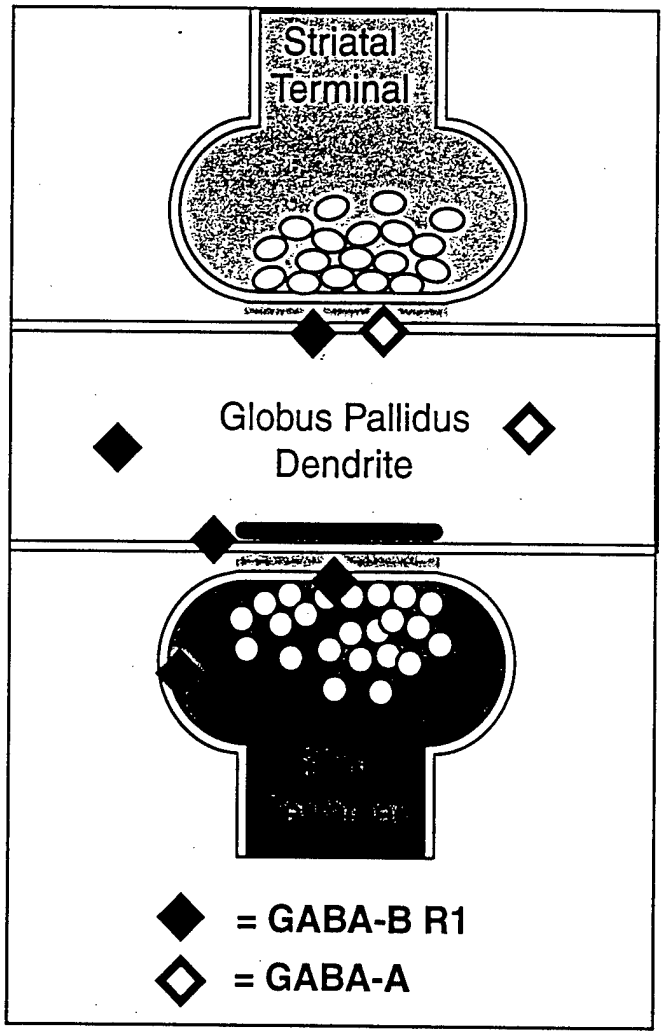


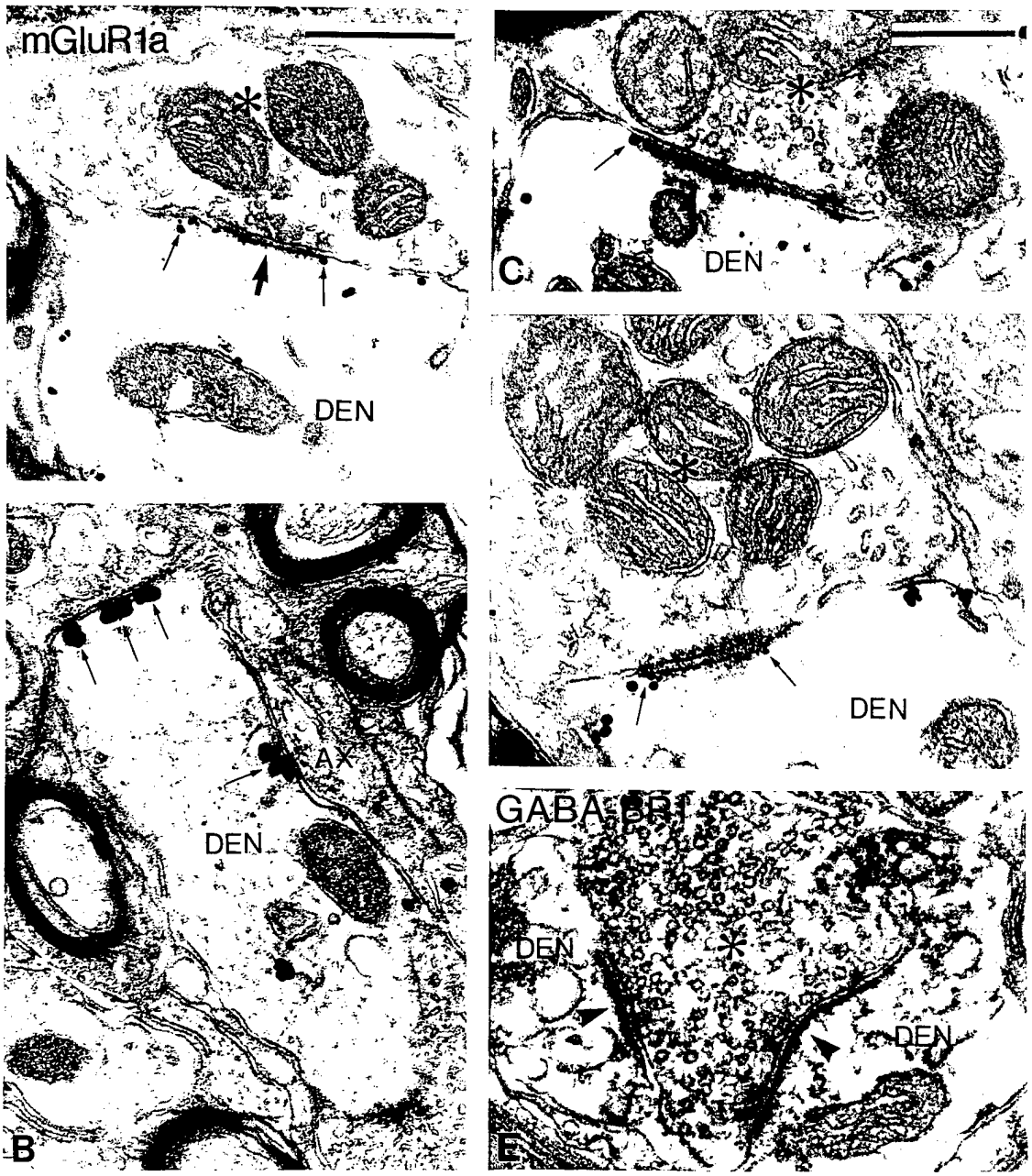


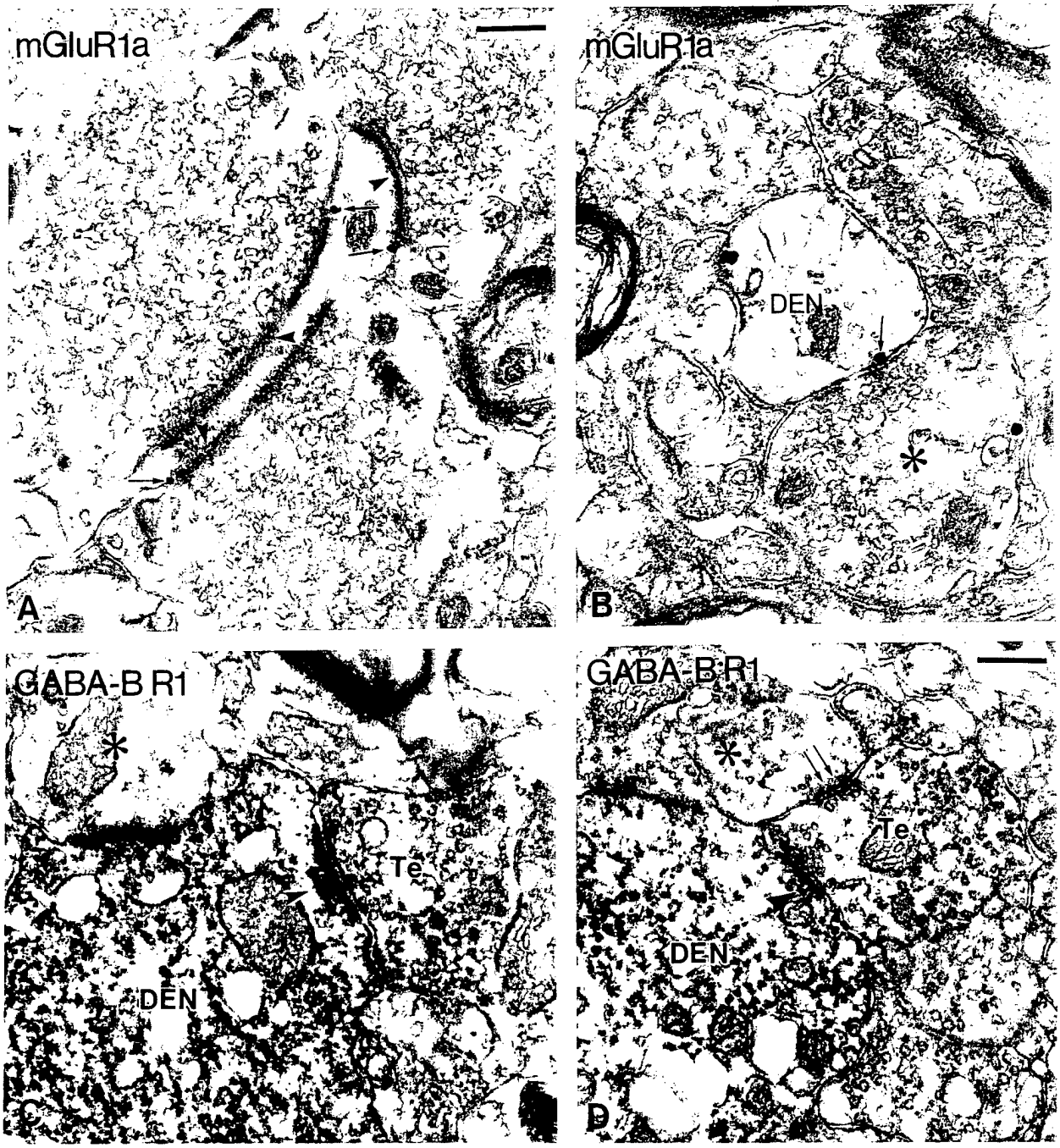
E


DEN
A
DEN
B
Striatal
Terminal
Globus Pallidus
Dendrite
C


GABA-A
A
DEN
GABA-BR1
B
DEN
GABA-BR1
C
DEN
GABA-BR1
D
DEN


Striatal
Terminal
Globus Pallidus
Dendrite
= GABA-B R1
= GABA-A


mGluR1a
DEN
C
DEN
DEN
A
DEN
B
GABA BR1
DEN
DEN
E


mGluR1a
A
mGluR1a
DEN
B
GABA-B R1
Te
DEN
C
GABA-B R1
Te
DEN
D